THE SEGREGATION OF *OENOTHERA BREVISTYLIS* FROM CROSSES WITH *OENOTHERA LAMARCKIANA*[1]

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INTRODUCTION

Genetical work upon the Oenotheras has developed a mass of data which has not as yet been interpreted in simple terms. There is the phenomenon called by DE VRIES and his followers "mutation," most strikingly illustrated in the appearance of marked variants from supposedly pure races or species when inbred. There are the peculiar patroclinous and matroclinous conditions presented in some F_1 generations and the subsequent reappearance of the parental characters in certain inter- and back-crosses. There is the production of two, three or even four types as the immediate result of certain crosses giving the so-called twin, triple and quadruple hybrids, where one would expect a uniform generation if the parental forms were homozygous. And there are a few cases reported in which the inheritance appears to follow a fairly simple Mendelian system which is at least clear as far as the fundamental principle of segregation is concerned although the ratios may be somewhat irregular.

[1] Genetical studies on Oenothera IX.

The writer has in recent years (DAVIS 1915 b, 1916 a, b, 1917) contended that these peculiarities of Oenothera genetics are due at least in large part if not wholly to a wide-spread genetical impurity general to the species and races of this group of plants and also to the high degrees

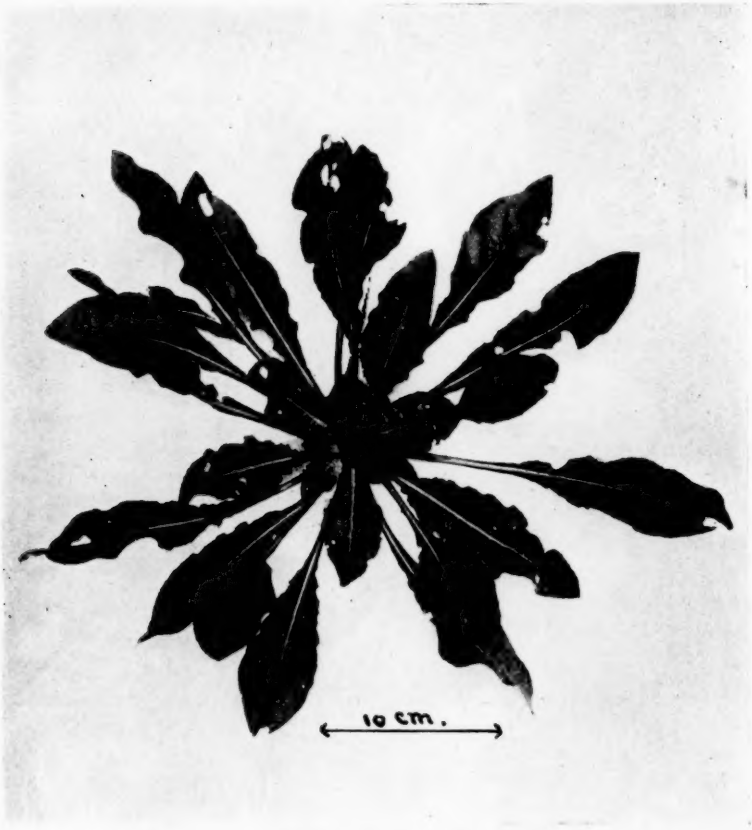

FIGURE 1.—Rosette of *Lamarckiana,* leaves mostly acute-pointed. Contrast with figure 2, *brevistylis.*

of gametic and zygotic sterility which makes it seem probable that our cultures generally represent imperfectly the behavior which would be expected if in supposedly complex material all of the gametes and zygotes were viable. Large proportions of the seeds of Oenothera through delayed germination unquestionably have not recorded their potential

products in the cultures of past years and only recently have methods been developed (DE VRIES 1915, DAVIS 1915 a) which make certain the germination of all or almost all of the viable seeds and permit of an examination of the residue of sterile structures. With the development of more perfect technique in the germination of seeds and in the establishment of cultures we may in the future hope for more exact data.

The hypothesis which largely forms the basis of the writer's present

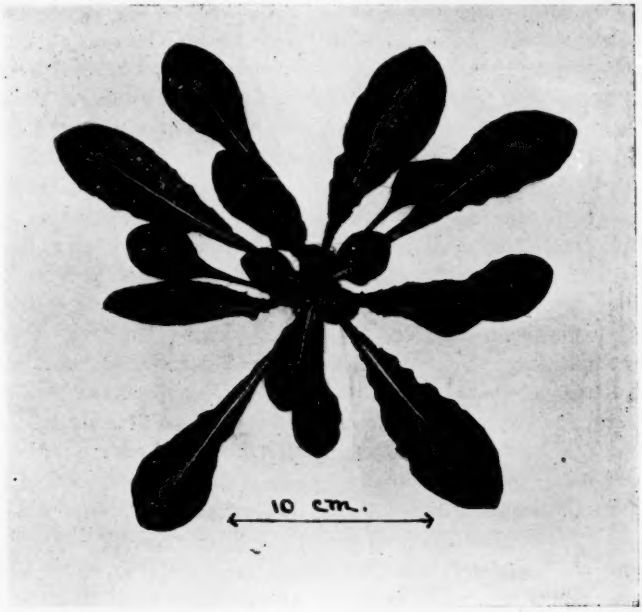

FIGURE 2.—Rosette of *brevistylis*, leaves obtusely pointed. Contrast with figure 1, *Lamarckiana*.

attitude (DAVIS 1915 b) postulates the existence in the Oenotheras of numerous impure or hybrid species, heterozygous in their germinal constitution, but which breed fairly true because for the most part only those gametes and zygotes are viable which may reproduce the parent type. Thus extensive gametic and zygotic sterility may be conceived to hold a hybrid line to a habit of relatively true breeding even though its germinal constitution remain heterozygous. The elimination through sterility of all gametes and zygotes excepting those which may carry

forward the heterozygous constitution of a hybrid may give an appearance of genetic purity which is the reverse of the facts. True breeding is not the final test of a pure species. Animals and plants must be shown to breed true because their germinal constitution permits the reduction divisions to give only identical products, except when sex differentiation is present, and the gametes must therefore be uniform for genetical factors that are not associated with sex. As the writer has emphasized in former papers no material of Oenothera has as yet passed the tests (DAVIS 1915 b) which justify our acceptance of it as representative of a pure species.

I do not care at this time to discuss in detail the bearing of this hypothesis of the impure species in its relation to the problems of Oenothera genetics but it may be remarked (1) that by the hypothesis most "mutations" may prove to be more readily interpreted as products of diverse gametic combinations from heterozygous material and that their presence in greater or less proportions depends upon their nature and is subject to the effects of sterility, and (2) that twin, triple and quadruple hybrids indicate that one or both of the parents cannot be homozygous since they are regularly producing different types of gametes which in these particular crosses prove to be fertile with one another.

Of the Oenothera material available for observations on possible Mendelian inheritance there is, as so far described, perhaps none more satisfactory for the characters concerned or more simple in its genetical nature than *Oe. brevistylis* crossed with *Oe. Lamarckiana.* The behavior of these hybrids gave the first suggestion of Mendelian system in Oenothera genetics and the only published studies upon the material have been those of Professor DE VRIES (1901-'03, Vol. 2, pp. 157, 159, 429-442; 1913, pp. 188-190) who made crosses of *brevistylis* not only with *Lamarckiana,* but with certain "mutants" from *Lamarckiana* and with some other species.

Other studies on Oenothera have described behavior apparently Mendelian in character. GATES (1915 a, p. 226) through a series of papers reached the conclusion that the peculiar pigmentation of *Oenothera rubricalyx* is dominant in crosses and holds that *rubrinervis* segregates from heterozygous *rubricalyx* in proportions approximating a 1 : 3 ratio. To his interpretations SHULL (1914) takes exception but GATES (1915 b) in a later paper reaffirms his position. DE VRIES (1915) reports the isolation of a heterozygous line of *gigas* that gave in one generation about 18 percent dwarfs, about 25 percent pure *gigas,* and about 57 percent of a *gigas*-like form, similar to the parent, which when selfed

proved itself to be heterozygous by again throwing about 21 percent of dwarfs.

DE VRIES reported the characters of *brevistylis* to be recessive in the F_1 generations of its crosses and then to appear in the F_2 generations in proportions fairly close to 25 percent of the progeny, which would be the expected Mendelian ratio for a simple monohybrid situation. My observations support the conclusions of DE VRIES. I have, however, data on a number of inter- and back-crosses not made by DE VRIES, and the cultures of the past two years have been grown from seed forced to complete germination. Consequently we have now before us considerably more data and that probably more reliable because of the methods of seed germination employed. There is, nevertheless a range of variation from expectations in the ratios of *brevistylis* segregates, the reasons for which are not at present clear. This matter will be taken up in the discussion.

METHODS OF CULTURE

The cultures of 1916 and 1917 were from seeds handled in the following manner. The entire contents of seed capsules were placed in vials half-filled with water and allowed to soak for 24—48 hours. The vials were then placed uncorked in a metal case made of iron pipe the cover of which can be fastened air tight by bolts. There is a pressure gauge attached to the cover and at the bottom of the case is an inlet tube such as is fitted into an automobile tire. Air was then forced into the case with an automobile pump until the pressure registered 100 pounds. The seeds in the water were left in the case under this air pressure for 24-36 hours. The contents of the vials were then poured over pads of filter paper in Petri dishes and the seeds evenly distributed. In 1916 the Petri dishes were placed in an incubator at temperatures from 28-31° C, but later experiments showed that germination in this material occurs quickly and completely when the Petri dishes are kept in a hothouse and I am inclined to think that some variations in temperature may favor rapid germination. It is surprising to see how quick may be the response in warm sunny weather when the temperature of a hothouse runs up high in the day time.

As rapidly as the seeds germinated they were removed to other Petri dishes and laid on wet pads of filter paper until the seedlings had so developed that they could be readily handled with the forceps and set into earth. The germinated seeds were counted and the seedlings were planted in rows in square earthen seed pans and any later losses through

death were thus readily noted. From the seed pans the young plants were transplanted to small pots. Seed-sterile soil was used in all cultures while in the hothouse. Cultures started in January and February produced strong rosettes by May which when set in the garden developed mature plants by early summer.

When, after 5-6 weeks, germination of the seeds seemed complete the

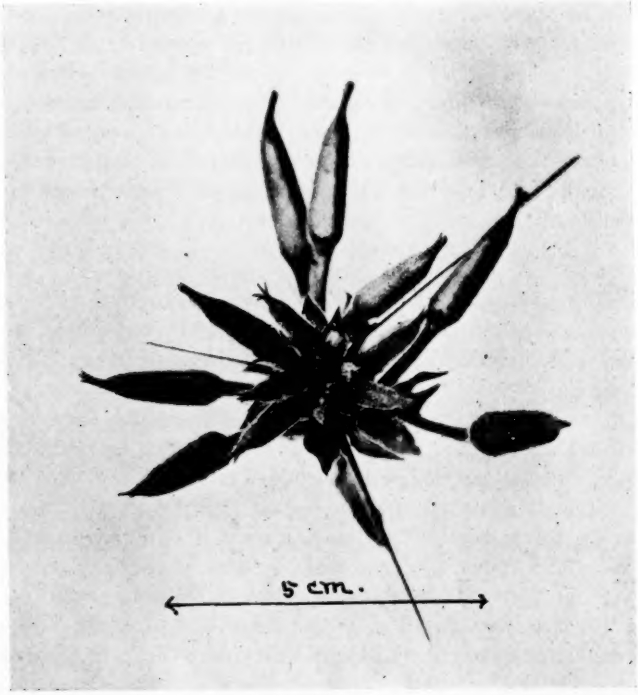

FIGURE 3.—Inflorescence of *Lamarckiana* viewed from above, showing narrower bud cones and attenuate sepal tips. Contrast with figure 4, *brevistylis*.

residue of ungerminated structures were tested for contents by pinching each seed with strong forceps. Most of the structures were found to be entirely empty, but a few contained small amounts of material readily squeezed out by the pressure of the forceps. Very rarely were any ungerminated seeds found, as shown by the presence of embryos. This residue of sterile structures arranged, dried, and fastened by shellac or glue on a piece of filter paper presented a record which together with

the number of germinated seeds enabled me to determine very exactly the percentage of seed fertility.

It is interesting to note in the tables how much higher in general is the percentage of germination obtained by this method of culture over the

FIGURE 4.—Inflorescence of *brevistylis* viewed from above, showing stouter bud cones and shorter sepal tips. Contrast with figure 3, *Lamarckiana*.

old practice of sowing seeds in the earth. The satisfaction of handling cultures with complete germination, thus giving accurate data on the percentage of seed sterility, is well worth the trouble involved and indeed is quite necessary where accuracy of results, so essential to Mendelian studies, are to be obtained in the group of the Oenotheras.

A COMPARISON OF THE PARENT SPECIES, *OENOTHERA LAMARCKIANA* AND *OE. BREVISTYLIS*

Although *Oenothera brevistylis* has never been observed in the lines of *Lamarckiana* cultivated by DE VRIES or derived from his material, its origin from this plant seems quite certain. It was reported by SCHOUTEN (1908) in a culture of *Lamarckiana* from commercial seeds, 6 plants of *brevistylis* among 502 *Lamarckiana* with 7 *lata*, 1 *nanella*, 3 *gigas* and 1

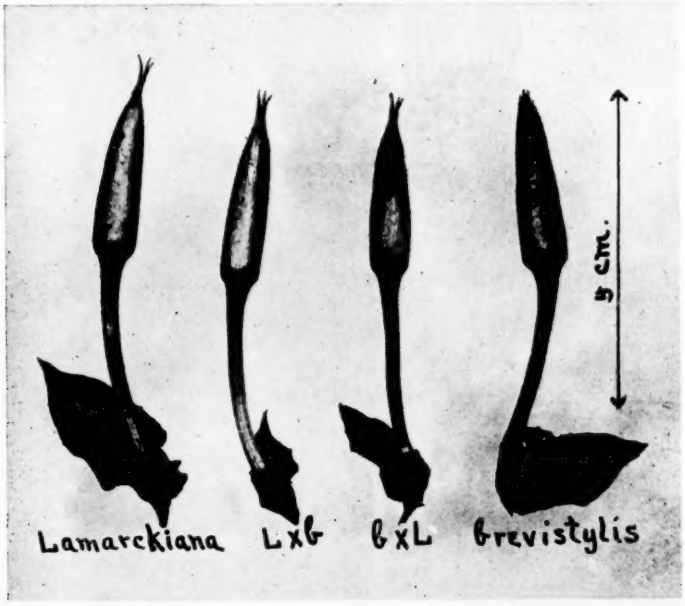

FIGURE 5.—Buds of *Lamarckiana*, *brevistylis*, and of the F_1 hybrids. Compare the sepal tips, the relative width of bud cones and the relative width of bracts. Note the contrasts between *Lamarckiana* and *brevistylis* and the high degree of dominance of *Lamarckiana* characters shown in the hybrids.

rubrinervis (ref. GATES 1915 a, p. 90). *Oenothera brevistylis* was discovered by DE VRIES in 1886 in the abandoned field at Hilversum growing among the plants of *Lamarckiana* and is known to have maintained itself in this locality for seventeen years. Although *brevistylis* sets very little selfed seed the fact that it maintains itself under suitable conditions is not remarkable since the plant pollinates *Lamarckiana* readily and the type segregates out perfectly in the F_2 generations from both of the reciprocal F_1 hybrids, from the double reciprocals, and from the back crosses of the F_1 hybrids with *brevistylis*. Furthermore, like a re-

cessive, which it is, *brevistylis* breeds perfectly true when selfed, although very small amounts of seed are produced even on favorable plants.

Oenothera brevistylis has been thoroughly described by POHL (1895) and DE VRIES (1903, p. 429). It differs from *Oe. Lamarckiana* in a number of characters so closely associated that so far as I know they have always been observed to be inherited as a group. These characters are contrasted in the following table.

Oenothera Lamarckiana	*Oenothera brevistylis*
Mature rosettes. Leaves acute-pointed (fig. 1).	Mature rosettes. Leaves obtuse-pointed (fig. 2).
Bracts. Narrower and acute-pointed (figs. 3 and 5).	Bracts. Broader and less pointed (figs. 4 and 5).
Buds. Longer 8—9 cm, their cones narrower, and the sepal tips attenuate, 7—8 mm long (figs. 3 and 5).	Buds. Shorter 5.5—6 cm, their cones more stout, and the sepal tips short, 1—2 mm long (figs. 4 and 5).
Style. Long, projecting 3—6 mm above the tips of the anthers (fig. 6).	Style. Short, generally terminating near the top of the calyx tube (fig. 6).
Stigma lobes. Well developed, 5—6 mm long (fig. 6).	Stigma lobes. Short and frequently rudimentary (fig. 6).
Ovary. Inferior, the ovules lying well below the insertion of the calyx tube (fig. 6).	Ovary. Partly superior, extending slightly above insertion of the calyx tube (fig. 6).
Capsules. Stout, 18—25 mm long, ripening from 100-350 seeds.	Capsules. Slender, scarcely longer than the ovaries, 9—11 mm, ripening no seeds or very few.

Plants of *Oenothera brevistylis* may be selected in the rosette stage by the practiced eye, but, as DE VRIES has pointed out, they do not stand out conspicuously among plants of *Lamarckiana* until the inflorescence begins to develop, when the broader and less pointed bracts and the very short sepal tips present sharp and clear differentiating characters. The absence of large capsules further distinguishes an older plant of *brevistylis* and the much longer flowering season is probably the result of its almost complete failure to ripen seed, thus conserving to the plant energy for a more extended period of flowering and vegetative growth.

Two races of *Oenothera Lamarckiana* have been employed in the crosses with *brevistylis*. *Lamarckiana* S came to me as a cross-bred

race developed by SHULL from nine lines of *Lamarckiana* originally obtained by DE VRIES from the field in Hilversum. I received seeds of *Lamarckiana* S (SHULL's culture 092.2) in 1911 and have carried a selfed line through six generations from that date. *Lamarckiana* M is a line received directly from DE VRIES and grown in my garden since 1910. These two lines are quite indistinguishable in their morphology, such differences in the measurements of their organs being too small to catch the eye, but they differ in their seed production and seed fertility. *Lamarckiana* M gives a yield of 200-350 seeds per capsule and the tested

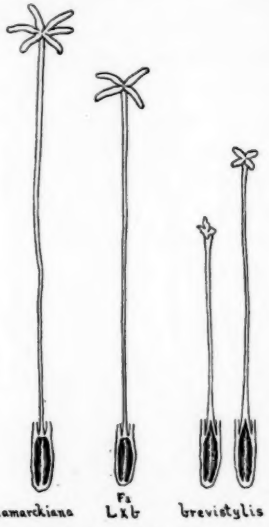

FIGURE 6.—Ovaries, styles and stigmas of *Lamarckiana, brevistylis* and the F_1 hybrid. Compare the relative lengths of the style, the degree of development of the stigma lobes and the extent of the ovarial cavity. Note the contrasts between *Lamarckiana* and *brevistylis* and the high degree of dominance of *Lamarckiana* characters shown in the hybrid.

germination in the last three generations (1915-17) has been respectively 32, 36 and 36 percent. *Lamarckiana* S develops from 100-250 seeds per capsule and the tested germination for the same years was 26, 30 and 31.8 percent. Each germination test was based on the contents of two large-sized capsules.

My material of *brevistylis* came to me through seeds set by an F_1 generation of *Lamarckiana* × *brevistylis* kindly sent to me by Professor DE VRIES. Although these seeds by the label on the package were

collected in 1907, I obtained germination and a culture of the F_2 generation in 1913. The culture gave the usual clean segregation of *brevistylis* and *Lamarckiana* characteristic of the second generation of this cross. One of the *brevistylis* plants was the parent of the earlier crosses with *Lamarckiana* described in this paper from which other plants of *brevistylis*, segregated in F_2 generations, were obtained for later crosses and for the selfed line of *brevistylis* started in 1916.

THE F_1 RECIPROCAL CROSSES

It is an easy matter to pollinate *Lamarckiana* by *brevistylis* and this cross was early made by DE VRIES who described the characters of the F_1 hybrid. The pollen of *brevistylis* is formed in abundance but as in *Lamarckiana* about 50 percent of the grains are shriveled; the good pollen, however, appears to be as effective as that of *Lamarckiana*. In general it may be said that the characters of *Lamarckiana* are strongly dominant over those of *brevistylis*. In the F_1 hybrid the rosette leaves are acute-pointed, the bracts narrow and acute-pointed, the sepal tips attenuate, the style long with well developed stigma lobes, the ovary inferior, the capsules large and ripening numerous seeds (180-220). I have made this cross employing both *Lamarckiana* S and *Lamarckiana* M as the seed-bearing parent and have found the germination percentage of *Lamarckiana* M $\times$ *brevistylis* (culture 16.14) to be 27.7 percent (two capsules tested with 360 seed-like structures) which is below the tested germination, 32-36 percent, for *Lamarckiana* M.

So similar is the F_1 hybrid of *Lamarckiana* $\times$ *brevistylis* to *Lamarckiana* that neighboring cultures cannot be distinguished excepting when the measurements of strongly contrasted characters are critically compared. Thus the style of the F_1 hybrid may not extend so far above the tips of the anthers as in *Lamarckiana* and occasionally plants are found with some of the flowers presenting stigma lobes midway between the anther tips and the top of the calyx tube. These occasional shorter-styled plants presented other characters somewhat intermediate between *brevistylis* and *Lamarckiana*. Their foliage leaves and bracts were broader than those of *Lamarckiana*, the bud cones were thicker and the sepal tips shorter, but the stigma lobes, while shorter, were not deformed and the yield of seed was good although smaller than that of *Lamarckiana*. The F_2 generations from two of these shorter-styled plants (14.15 *y* and 14.15 *z*) presented a clear segregation of *brevistylis* and *Lamarckiana* types (table 1) and I am forced to conclude that the peculiarities of the F_1 parents did not signify genetical differences from the usual F_1

hybrids of *Lamarckiana* × *brevistylis*. These plants were apparently instances of imperfect dominance where strong dominance is the rule.

The reciprocal cross *brevistylis* × *Lamarckiana* seems not to have been reported by DE VRIES. It can be made and a small yield of seeds obtained by pollinating a large number of *brevistylis* flowers selecting those with better-developed stigma lobes. Thus after pollinating many flowers of *brevistylis* by *Lamarckiana* S in 1915 I obtained a total of 30 small capsules which altogether yielded only 85 seed-like structures from which 19 seedlings appeared in 1916. Pollinations of *brevistylis* by *Lamarckiana* M made in 1916 gave 58 seed-like structures from 18 capsules and in 1917 (culture 17.59) a total of 24 seedlings, a germination of 41 percent. This was higher than the germination of 27.7 percent shown by the reverse cross *Lamarckiana* M × *brevistylis* (culture 16.14), but the small numbers of seeds and seedlings obtained in the cross *brevistylis* × *Lamarckiana* M makes it unsafe to assume that 41 percent is very close to the correct germination proportions.

Plants of the F_1 hybrids *brevistylis* × *Lamarckiana* are so similar to plants of the reciprocals *Lamarckiana* × *brevistylis* that I know of no distinguishing differences. As in the reciprocal cross the characters of *Lamarckiana* are strongly dominant in the F_1 generation of *brevistylis* × *Lamarckiana*.

THE F_2 GENERATION FROM THE F_1 OF *LAMARCKIANA* × *BREVISTYLIS*

My data on this generation is based on families derived from eleven sister plants in an F_1 generation (culture 14.15) of the cross *Lamarckiana* S × *brevistylis*. The F_1 parent plants were designated 14.15 *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *y* and *z*. The F_2 families from 14.15 *b*, *c*, and *d* were grown in 1915 from seed sown in earth. The families from 14.15 *e* and *f* were grown by Dr. G. H. SHULL at Cold Spring Harbor, Long Island, in 1915, also from seed sown in earth. The families from 14.15 *g*, *h*, *i*, and *j* were grown in 1916 from seed experimentally germinated as described above under Methods of Culture, the seedlings being set in pans. The F_1 plants 14.15 *y* and *z* were of the type with styles on some of the flowers midway between the tips of the anthers and the top of the calyx tube (see p. 511); from seed of such flowers selfed two families were grown in 1915, the seeds being sown in earth.

All of the F_2 families presented a perfectly sharp segregation of *brevistylis* plants from *Lamarckiana* but with somewhat irregular ratios. The ratios, however, are close enough to the simple Mendelian 1 : 3 to justify the hypothesis that *brevistylis* is a simple recessive from *Lamarck-*

iana and that the cross for the characters concerned presents a monohybrid situation. The data are assembled in table 1 and will be discussed in the succeeding paragraphs.

A consideration of the data presented in table 1, taken as a whole, is only possible with respect to a few general conclusions because some of the cultures were from seeds sown in earth and others from seeds experimentally forced to a complete expression of their viability. It should be noted, however, that except for an occasional *oblonga*-like form or some dwarf rosettes, including *albida*, the plants were either *brevistylis* or *Lamarckiana*. The plants recorded as having died were usually weak seedlings which succumbed in the seed pans. There was a wide variation in the percentages of germination even when the wet seeds were subjected to pressure and then placed in Petri dishes. It might seem that the eleven sister plants of the F_1 varied in their power to produce viable seed and were therefore in this respect genetically different but it is possible that physiological conditions surrounding the individual plants led to these varying outputs of viable seeds. The data throw no light on this problem which can only be approached by physiological experiment. In three families (cultures 15.15 *c*, 16.15 *h* and 16.15 *i*) the ratios of *brevistylis* to *Lamarckiana* (1 : 15.5, 1 : 9.8 and 1 : 9) were very much less than the 1 : 3 ratios of our hypothesis, and in these cultures the germination percentages of 10.9, 19 and 15.8 were much below the higher percentages suggesting a correlation between low seed viability and smaller proportions of *brevistylis*. However, cultures SHULL 14169 and 16.15 *j* with rather low ratios of *brevistylis* to *Lamarckiana*, 1 : 6.5 and 1 : 5.4, but high percentages of germination, 31.2 and 33.3, did not support the hypothesis. Culture 15.15 *d*, from earth-sown seeds, with its very low percentage of germination (5.37) was too small a family to have much value in a discussion.

In a general consideration of the table I would emphasize the facts of a clear segregation of *brevistylis* from *Lamarckiana* in proportions for six of the eleven families close to the monohybrid ratio of 1 : 3. As the paper proceeds we shall find clearer evidence in support of the view that *brevistylis* is a simple recessive differing from *Lamarckiana* in a group of characters which are associated in inheritance.

Of particular interest are the data from cultures 15.41 and 15.42 since the F_1 parent plants bore some flowers with styles midway between the tips of the anthers and the top of the calyx tube (see p. 511) although in other respects resembling *Lamarckiana*. The genetical behavior of these two plants was quite the same as that of the sister plants

TABLE 1

Segregation of brevistylis from Lamarckiana in the F_2 from the F_1 of Lamarckiana S × brevistylis.

Culture	F_1 parent plant	Seeds sown	Sown in	Percentage of germination	Number of *brevistylis*	Number of *Lamarckiana*	Other plants in the culture	Ratio of *brevistylis* to *Lamarckiana* and other plants
15.15 *a*	14.15 *a*	577 (4 capsules)	Petri dish	30.6*	—*	—		—
15.15 *b*	14.15 *b*	346 (4 capsules)	Earth	17	14	44		1 : 3.1 (1 plant died)
15.15 *c*	14.15 *c*	321 (4 capsules)	Earth	10.9	2	33		1 : 15.5 (2 plants died)
15.15 *d*	14.15 *d*	391 (4 capsules)	Earth	5.37	5	11		1 : 2.2 (5 plants died)
14169 SHULL	14.15 *e*	173	Earth	31.2	7	45	1 rosette	1 : 6.5 (1 plant died)
14177 SHULL	14.15 f	230	Earth	17.4	8	30	1 rosette	1 : 3.8 (1 plant died)
16.15 *g*	14.15 *g*	293 (2 capsules)	Petri dish	37.8	25	79	2 *oblonga*-like 1 narrow-leaved dwarf rosette	1 : 3.28 (4 plants died)
16.15 *h*	14.15 *h*	297 (2 capsules)	Petri dish	19	5	48	1 *oblonga*-like	1 : 9.8 (3 plants died)
16.15 *i*	14.15 *i*	208 (2 capsules)	Petri dish	15.8	3	26	1 *albida*	1 : 9 (3 plants died)
16.15 *j*	14.15 *j*	270 (2 capsules)	Petri dish	33.3	13	70	1 *albida*	1 : 5.4 (6 plants died)
15.41	14.15 *y*	163 (3 capsules)	Earth	34.3	16	36		1 : 2.2 (4 plants died)
15.42	14.15 *z*	97 (3 capsules)	Earth	27.8	7	19		1 : 2.7 (1 plant died)

* This culture was merely a test of the percentage of seed germination.

in the F_1 which showed a dominance of *Lamarckiana* characters, that is to say the segregation into *brevistylis* and *Lamarckiana* was just as sharp. In culture 15.41 only 5 of the 36 *Lamarckiana* plants were observed to produce occasional shorter-styled flowers and in culture 15.42 only 4 of the 19 *Lamarckianas* presented a similar behavior. Thus the tendency to develop shorter-styled flowers seemed no more marked in the *Lamarckiana* segregates of the F_2 than in the F_1 parent plants and I see no reason to believe that the peculiarity depends upon genetical factors different from those present in the usual F_1 hybrids of *Lamarckiana* × *brevistylis*. It is a peculiarity so variable in the degree of its expression and affecting so small a proportion of the flowers on a plant that its appearance seems more likely to be due to physiological causes. I found no material in the F_2 which would lead me to hope that the shorter-styled conditions might be fixed through selection.

A family in the F_3 (culture 16.3) from a selfed *Lamarckiana* plant in the F_2 of culture 15.15 *b* gave from 64 seedlings a total of 61 *Lamarckianas* and 2 narrow-leaved dwarfs indicating that this plant was pure *Lamarckiana*. Lack of garden space has so far prevented my testing a complete set of *Lamarckiana* plants from an F_2 generation. By our hypothesis 1/3 of the plants in such a group should be pure *Lamarckiana* and 2/3 should prove to have the constitution of the F_1 hybrid.

THE F_2 GENERATION FROM THE F_1 OF *BREVISTYLIS* × *LAMARCKIANA*

The records of the F_2 generations from *brevistylis* × *Lamarckiana* S are more satisfactory than the F_2 from the reciprocal F_1 cross described in the previous section. This is chiefly for the reason that all of the cultures were grown from wet seed subjected to pressure and germinated in Petri dishes, a treatment which resulted in the complete germination of all viable seeds. The four F_2 cultures were from F_1 plants in two different families and were related as sister plants as follows—16.1 *a*, I-3 and 16.1 *a*, II-3; 16.1 *b*, I-2, and 16.1 *b*, I-4. Therefore in a consideration of the genetical data, given in table 2, the F_2 cultures for purposes of comparison should be considered in pairs—17.51 and 17.52, 17.53 and 17.54.

As in the F_2 cultures previously described the segregation of *brevistylis* plants from *Lamarckiana* was perfectly sharp but the ratios were irregular. The most extreme range of variation, ratio 1 : 6.2 (culture 17.53) and ratio 1 : 2.5 (culture 17.54) is not as great as that shown in the cultures from reciprocal F_1 plants (see table 1) where the lowest ratio was 1 : 15.5 and the highest 1 : 2.2, both being from earth-sown cul-

TABLE 2

Segregation of brevistylis from Lamarckiana in the F_2 from the F_1 of brevistylis × Lamarckiana S.

Culture	F_1 parent plant	Seeds sown	Sown in	Percentage of germination	Number of *brevistylis*	Number of *Lamarckiana*	Other plants in the culture	Ratio of *brevistylis* to *Lamarckiana* and other plants
17.51	16.1 *a*, I-3	332 (2 capsules)	Petri dish	28.9	14	79		1 : 5.6 (3 plants died)
17.52	16.1 *a*, II-3	296 (2 capsules)	Petri dish	37.1	28	69	3 *oblonga*-like 1 *albida* 1 broad-leaved dwarf rosette	1 : 2.6 (8 plants died)
17.53	16.1 *b*, I-2	313 (3 capsules)	Petri dish	30.9	13	76	2 *oblonga*-like 1 *laevifolia*-like 1 *albida* 1 broad-leaved dwarf rosette	1 : 6.2 (3 plants died)
17.54	16.1 *b*, I-4	514 (3 capsules)	Petri dish	35.7	48	117	2 *oblonga*-like 1 *laevifolia*-like 2 *elliptica* 1 round-leaved dwarf rosette	1 : 2.5 (13 plants died)

TABLE 3

Segregation of brevistylis from Lamarckiana in double reciprocal crosses.

Culture	Seeds sown	Sown in	Percentage of germination	Number of *brevistylis*	Number of *Lamarckiana*	Other plants in the culture	Ratio of *brevistylis* to *Lamarckiana* and other plants
17.61 $(L \times b) \times (b \times L)$	522 (3 capsules)	Petri dish	29.1	29	110	6 *oblonga*-like 2 *albida*	1 : 4 (5 plants died)
17.62 $(b \times L) \times (L \times b)$	398 (3 capsules)	Petri dish	32.6	36	87	1 *oblonga*-like 1 *lata* 1 narrow-leaved dwarf rosette	1 : 2.5 (4 plants died)

tures. This closer approximation of the ratios is perhaps due to the fact that all of the seeds were fully germinated by the method of treatment with pressure.

It is interesting that the two families (cultures 17.51 and 17.53) with ratios of *brevistylis* to *Lamarckiana* much less than 1 : 3 (being 1 : 5.6 and 1 : 6.2) were families with lower percentages of germination (28.9 and 30.9) than in the cultures with which they were paired (cultures 17.52 and 17.54). This suggests very clearly a correlation between low seed viability and smaller proportions of *brevistylis*. It is noteworthy that in table 2 the higher ratios of *brevistylis* to *Lamarckiana* (cultures 17.52 and 17.54) are very close to 1 : 3 which was also true of the data assembled in table 1.

A larger number and greater variety of "mutants" were noted in these cultures than in those recorded in table 1, a fact perhaps due to complete germination. The plants listed as having died, generally refer to weak seedlings unable to live long.

THE DOUBLE RECIPROCAL CROSSES

Crosses were made both ways between a plant in the F_1 of *Lamarckiana* M × *brevistylis* (16.14 VI-3) and a plant in the F_1 of *brevistylis* × *Lamarckiana* S (16.1 *b* I-4) giving what DE VRIES has termed double reciprocal crosses. The same *brevistylis* stock was in both of the F_1 plants employed, but it happened that one of these F_1 hybrids was concerned with the race of *Lamarckiana* M and the other with the race *Lamarckiana* S. Therefore the double reciprocal crosses do not involve exactly equivalent lines of *Lamarckiana,* although, as has been stated, *Lamarckiana* M and S do not differ in their systematic characters and both lines run back to plants secured by DE VRIES at Hilversum.

The two cultures were 17.61, (*Lamarckiana* M × *brevistylis*) × (*brevistylis* × *Lamarckiana* S), and 17.62, (*brevistylis* × *Lamarckiana* S) × (*Lamarckiana* M × *brevistylis*). On an assumption that the same types of fertile gametes are formed through both pollen and ovules of the parent F_1 hybrid plants the generations from a double reciprocal cross should give the same segregation as F_2 generations from either F_1 parent of the cross. Thus the F_1 *Lamarckiana* × *brevistylis* and the F_1 *brevistylis* × *Lamarckiana* by our hypothesis develop gametes *b* and *L,* and when each is selfed the combinations in the F_2 generations under conditions of uniform fertility would be 1 *bb* : 2 *bL* : 1 *LL* or the phenotypic ratio of 1 *brevistylis* : 3 *Lamarckiana.* These same F_1 hybrids when crossed to give double reciprocals would therefore be expected to

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present the same segregation, namely 1 *brevistylis* : 3 *Lamarckiana,* since each develops the same types of gametes. The breeding data of the double reciprocal crosses are given in table 3 (p. 516).

The expectations of our hypothesis were supported by the breeding results since in both double reciprocals the proportion of *brevistylis* to *Lamarckiana* was close to the ratio 1 *brevistylis* : 3 *Lamarckiana.* The departure of the ratios in these two cultures of double reciprocals from the 1 : 3 of our hypothesis is perhaps not greater than might be expected in small cultures, but it is interesting to find again a possible correlation of a lower germination percentage (29.1 in culture 17.61) with a smaller proportion of *brevistylis* (1 : 4). As in the cultures previously described, the plants listed as having died usually refer to weak seedlings.

BACK CROSSES OF THE F_1 RECIPROCAL HYBRIDS (*LAMARCKIANA* × *BREVISTYLIS*) AND (*BREVISTYLIS* × *LAMARCKIANA*) TO THE PARENT STOCK

Back crosses of the two F_1 reciprocals (*Lamarckiana* × *brevistylis*) and (*brevistylis* × *Lamarckiana*) were made in all possible combinations with the parent stock giving the eight cultures listed below, to which DE VRIES's convenient descriptive terms of sesquireciprocal and iterative are also applied.

17.63	Iterative	(*Lamarckiana* M × *brevistylis*) × *brevistylis*
17.64	Sesquireciprocal	*brevistylis* × (*Lamarckiana* M × *brevistylis*)
17.65	Sesquireciprocal	(*Lamarckiana* M × *brevistylis*) × *Lamarckiana* M
17.66	Iterative	*Lamarckiana* M × (*Lamarckiana* M × *brevistylis*)
17.67	Sesquireciprocal	(*brevistylis* × *Lamarckiana* S) × *brevistylis*
17.68	Iterative	*brevistylis* × (*brevistylis* × *Lamarckiana* S)
17.69	Iterative	(*brevistylis* × *Lamarckiana* S) × *Lamarckiana* S
17.70	Sesquireciprocal	*Lamarckiana* S × (*brevistylis* × *Lamarckiana* S)

It should be noted that the two races of *Lamarckiana,* M and S, were concerned in the series, but that each back cross involved the same race. Breeding data for the eight crosses are given in table 4.

The results of the back crosses are exactly as would be expected from our hypothesis as far as the character of the progeny is concerned. Back crosses to *Lamarckiana* whether the pollen parent (cultures 17.65 and 17.69) or the ovule parent (cultures 17.66 and 17.70) gave progenies with no plants of *brevistylis,* thus showing the dominance of *Lamarckiana* so conspicuous in the F_1 generations. Back crosses to *brevistylis,* the recessive, gave perfectly clear segregation of *brevistylis* (cultures 17.63, 17.64, 17.67 and 17.68). Fertilization of *brevistylis* by the

TABLE 4

Back crosses of the F_1 reciprocal hybrids (Lamarckiana × brevistylis) and (brevistylis × Lamarckiana) to the parent stock.

Culture	Seeds sown	Sown in	Percentage of germination	Number of *brevistylis*	Number of *Lamarckiana*	Other plants in the culture	Ratio of *brevistylis* to *Lamarckiana* and other plants
17.63 $(L \times b) \times b$ Iterative	427 (2 capsules)	Petri dish	25.5	42	58	3 *oblonga*-like 1 *albida* 1 broad-leaved dwarf rosette	1 : 1.5 (4 plants died)
17.64 $b \times (L \times b)$ Sesquireciprocal	76 (15 capsules)	Petri dish	32.8	4	4		1 : 1 (17 plants died)
17.65 $(L \times b) \times L$ Sesquireciprocal	314 (2 capsules)	Petri dish	43.9	none	127	1 *oblonga*-like 2 *nanella* 1 *albida*	0 : 1 (7 plants died)
17.66 $L \times (L \times b)$ Iterative	244 (2 capsules)	Petri dish	25.8	none	58	1 *nanella*	0 : 1 (4 plants died)
17.67 $(b \times L) \times b$ Sesquireciprocal	295 (3 capsules)	Petri dish	35.5	36	61	1 *oblonga*-like 1 *rubrinervis* 1 narrow-leaved dwarf rosette	1 : 1.7 (5 plants died)
17.68 $b \times (b \times L)$ Iterative	37 (18 capsules)	Petri dish	37.8	1	8		1 : 8 (5 plants died)
17.69 $(b \times L) \times L$ Iterative	359 (3 capsules)	Petri dish	51.8	none	180	1 *laevifolia*-like 1 *lata*	0 : 1 (4 plants died)
17.70 $L \times (b \times L)$ Sesquireciprocal	513 (3 capsules)	Petri dish	33.5	none	165	3 *Lamarckiana*-like rosettes 1 *albida*	0 : 1 (3 plants died)

F_1 hybrids proved difficult of accomplishment because of its low seed fertility and the yield of seeds was very small. Thus culture 17.64 came from 76 seeds, the contents of 15 capsules, and culture 17.68 came from 37 seeds, the contents of 18 capsules. In both of these cultures the mortality among seedlings and young plants was very great (68 percent for culture 17.64 and 35.8 percent for culture 17.68) and because of this fact together with the small numbers in the cultures the ratios of *brevistylis* to *Lamarckiana*, 1 : 1 and 1 : 8, have little value for our discussion.

For the other two back crosses with *brevistylis* as the pollen parent, cultures 17.63 and 17.67, we have fairly large families and ratios of *brevistylis*, 1 : 1.5 and 1 : 1.7, which approximate the ratio of 1 : 1 expected in a cross of a monohybrid back to a recessive parent stock, when there are no complications of differential mortality among gametes or zygotes. It seems significant that the numbers of *brevistylis* fall below expectations and a failure of *brevistylis* to reach expected proportions or to go very slightly beyond these proportions is generally characteristic of the results recorded in tables 1, 2, and 3 as well as in table 4. For the cultures here described it seems possible that a mortality, higher for *brevistylis*, may be responsible for the departures from expected genetical ratios. This problem will receive further consideration in the discussion.

OBSERVATIONS ON A SELFED LINE OF *OENOTHERA BREVISTYLIS*

As previously stated my material of *brevistylis* came to me from Professor DE VRIES through seeds of an F_1 *Lamarckiana* × *brevistylis*. From these seeds a culture was grown in 1913 which showed the usual F_2 segregation into *brevistylis* and *Lamarckiana* (culture 13.15). Pollination of *Lamarckiana* S from one of the *brevistylis* plants gave me in 1914 an F_1 generation (culture 14.15) and selfing eleven of these F_1 plants (14.15 *b, c, d, e, f, g, h, i, j, y* and *z*) made possible in 1915 and 1916 the F_2 cultures described in table 1.

Among the *brevistylis* plants in these F_2 families some were noted as having a much larger proportion of flowers with longer styles and more perfectly formed stigmas than seemed usual for *brevistylis* (fig. 7). Thus an opportunity was presented to test the possibility of improving the style length of *brevistylis* and its fertility by selfing flowers on selected plants showing a greater variation in the direction of normal Oenothera styles and stigmas. Four plants of *brevistylis* were chosen (15.15 *b*, I-1; 15.15 *b*, I-4; 15.15 *b*, II-5; 15.15 *d*, I-1), favorable flowers were self-pollinated and from their seed were grown the cultures of selfed *brevistylis* listed in table 5.

TABLE 5

Culture	Parent *brevistylis* plant	Seeds sown	Sown in	Percentage of seeds with contents	Number of seedlings	Plants reaching maturity, all *brevistylis*
16.2 *a*	15.15 *b*, I-1	55 15 capsules	Petri dish	45	21	15
16.2 *b*	15.15 *b*, I-4	130 21 capsules	Petri dish	46	45	43
16.2 *c*	15.15 *b*, II-5	78 14 capsules	Petri dish	35.9	18	18
16.2 *d*	15.15 *d*, I-1	33 5 capsules	Petri dish	48.5	16	16

FIGURE 7.—At the right, a flower of *brevistylis* with stigma at a much higher level than is usual for this species (plant 15.15 *b*, I-1). A selfed line of plants showing large proportions of such flowers is being carried along to test the possibility of improving through selection the style length and fertility of *brevistylis*.

TABLE 6

Performance in the first generation of 8 plants of brevistylis from selfed parents selected for their tendency to produce long-styled flowers.

Plants	Observations	Flowers observed	Long styles	Short styles	Percentage of long styles	Yield of seed from selfed flowers
16.2 *b*, II-3	July 18-Aug. 3	26	21	5	80	18 flowers selfed gave 10 shriveled capsules with 12 seeds
16.2 *b*, III-3	July 18-Aug. 2	16	9	7	56	12 flowers selfed gave 7 capsules with 18 seeds
16.2 *b*, VI-5	July 18-Aug. 4	24	15	9	62	16 flowers selfed gave 9 capsules with 19 seeds
16.2 *b*, VII-1	July 18-Aug. 2	16	13	3	81	18 flowers selfed gave 12 capsules with 36 seeds
16.2 *d*, I-2	July 18-Aug. 1	25	20	5	80	21 flowers selfed gave 11 capsules with 52 seeds
16.2 *d*, II-4	July 18-Aug. 2	15	13	2	86	17 flowers selfed gave 9 capsules with 31 seeds
16.2 *d*, IV-1	July 18-Aug. 5	22	21	1	95	21 flowers selfed gave 13 capsules with 55 seeds
16.2 *d*, IV-4	July 18-Aug. 3	19	19	0	100	14 flowers selfed gave 8 capsules with 104 seeds

TABLE 8

Performance in the second generation of brevistylis in a selfed line from parents selected for their tendency to produce long-styled flowers.

Plants	Observations	Flowers observed	Long styles	Medium styles	Short styles	Percentage of long styles	Yield of seed from selfed flowers
17.2, I-1	June 28-July 28	37	11	11	15	29.7	
*I-2	" 30- " 28	26	18	5	3	69.2	13 flowers selfed gave 2 capsules with 7 seeds
*I-3	" 28- " 28	31	17	11	3	54.8	10 flowers selfed gave 2 capsules with 5 seeds
I-4	" 28- " 28	37	14	8	15	37.8	
I-5	July 3- " 28	28	12	11	5	42.8	
*II-1	June 28- " 28	34	22	11	1	64.7	17 flowers selfed gave no capsules
II-2	" 28- " 28	37	16	12	9	43.2	
II-3	" 29- " 28	34	16	15	3	47	
II-4	" 30- " 28	27	6	9	12	22.2	
II-5	July 3- " 28	27	8	12	7	29.6	
*III-1	June 28- " 28	34	25	6	3	73.5	19 flowers selfed gave 2 capsules with 4 seeds
III-2	" 29- " 28	29	9	12	8	31	
*III-3	" 30- " 28	29	19	5	5	65.5	15 flowers selfed gave 6 capsules with 21 seeds
III-4	" 28- " 28	32	11	12	9	34.3	
III-5	July 2- " 28	27	2	10	15	7.4	
IV-1	June 28- " 28	33	13	7	13	39.3	
IV-2	" 29- " 28	32	3	17	12	9.3	
IV-3	" 29- " 28	35	7	14	14	20	
IV-4	" 29- " 28	26	4	6	16	15.3	
IV-5	July 1- " 28	29	11	11	7	37.9	
*V-1	June 30- " 28	31	19	9	3	61.3	24 flowers selfed gave 11 capsules with 54 seeds. Largest capsules 9-10 mm long
V-2	" 29- " 28	30	5	10	15	16.6	
V-3	" 30- " 28	34	10	13	11	29.4	
V-4	" 30- " 28	28	8	15	5	28.5	
V-5	July 3- " 28	28	3	10	15	10.7	
VI-1	" 1- " 28	27	6	10	11	22.2	
VI-2	June 29- " 28	32	4	14	14	12.5	
*VI-3	July 1- " 28	26	20	5	1	76.9	11 flowers selfed gave no capsules
VI-4	" 3- " 28	26	9	8	9	34.6	
*VII-1	" 2- " 28	28	21	6	1	75	18 flowers selfed gave no capsules
VII-2	" 2- " 28	25	9	12	4	36	
*VII-3	" 1- " 28	33	17	13	3	51.5	16 flowers selfed gave no capsules
VII-4	" 1- " 28	31	0	12	19	0	

These cultures of selfed *brevistylis* (16.2 *a*, *b*, *c* and *d*) presented a wide range of variation in their tendency to produce flowers with styles longer than is usual to *brevistylis*. Consequently the performance of individual plants was tested before making selections to self for the next generation. The test consisted in recording the length of style in a series of flowers opening along the main stem of a plant over a definite period of time. In these cultures the styles were recorded as *long* when they extended well above the top of the calyx tube and as *short* if the stigma was at or below the level of the calyx tube. The record of performance as observed for eight plants is given in table 6.

From its record of performance, as shown in table 6, plant 16.2 *d*, IV-4 was picked as the parent for a second generation of this selfed line of *brevistylis*. It set the usual small quantity of seeds when selfed, 104 seeds from 8 capsules, and from these there was grown in 1917 a culture with a record as follows:

TABLE 7

Culture	Parent *brevistylis* plant	Seeds sown	Sown in	Percentage of germination	Number of seedlings	Plants reaching maturity, all *brevistylis*
17.2	16.2 *d*, IV-4	104 8 capsules	Petri dish	34.6	36	33

The performance of the 33 plants in this second generation of selfed *brevistylis* with respect to their production of longer-styled flowers was recorded as in the case of the first generation of selfed *brevistylis* by observing for each plant a series of flowers as they opened over a certain length of the main stem. The classification was, however, modified so that three grades were noted instead of two, *long* when the styles reached the lower ends of the anthers or extended above, *medium* when the stigma lay between the lower ends of the anthers and top of the calyx tube, short when the stigma was at or below the top of the calyx tube. The record of the performance of this second generation of selfed *brevistylis* is given in table 8.

An examination of table 8 will show at once a very marked regression of the second generation of selfed *brevistylis* from the performance of its parent plant 16.2 *d*, IV-4 (table 6), which while under observation presented no short-styled flowers of our classification and a yield of 104 seeds from 8 capsules, an average of 13 seeds to a capsule. The plants making the best records in the second generation were *17.2, I-2; *I-3;

*II-1; *III-1; *III-3; *V-1; *VI-3; *VII-1; *VII-3. These plants were selfed by carefully selecting flowers with longer styles and better-developed stigmas but the results were disappointing as to the yield of seeds (see table 8). Some of the plants developed no seeds, the ovaries withering; others developed occasional small capsules with few seeds. The best yield was from plant *17.2, V-1, from which, following 24 selfings, 11 capsules were obtained with a total of 54 seeds, an average of about 5 seeds to a capsule. No plant in this second generation equaled the record of the parent in style length and seed production and most of the plants fell far behind its performance.

I expect to carry this selfed line through further generations selecting for length of style and for seed production, since fertility is of course necessary to progress of the study. The material presents a character (length of style and development of stigma lobes) highly variable and would seem to be excellent for a test of the power of selection to achieve results in a close-pollinated line.

DISCUSSION

Before taking up as a whole the data on the ratios of *brevistylis* plants segregated from the crosses with *Lamarckiana* the following facts should be remembered in their broad relations to the problem. (1) There is presented in the F_1 an almost perfect dominance of *Lamarckiana* characters when this plant is crossed with *brevistylis* or with an F_1 hybrid of *Lamarckiana* and *brevistylis*. (2) There is a perfectly clean segregation of *brevistylis* plants in the F_2 from the reciprocal F_1 generations of crosses between *brevistylis* and *Lamarckiana*, from the double reciprocal crosses $(L \times b) \times (b \times L)$ and $(b \times L) \times (L \times b)$, and from the back crosses of the F_1 hybrids to *brevistylis*. (3) *Brevistylis* shows itself to be a recessive, by breeding true, by the dominance of *Lamarckiana* in crosses, and by segregating from appropriate crosses in ratios approximating those to be expected of monohybrids.

Mention should first be made of other variants from *Lamarckiana* which appeared in the cultures besides *brevistylis*. They are listed in tables 1, 2, 3 and 4, and it is interesting to note that the earth-sown cultures quite failed to bring them out; they only appeared in cultures from seed forced to complete germination. Most of the variants were easily identified with "mutants" described by DE VRIES from *Lamarckiana*. Thus *albida* (9), *nanella* (3), *lata* (2), and *rubrinervis* (1) were readily recognized. A type close to *oblonga*, but generally larger, was most common (22) and a narrow-leaved form similar to *laevifolia* (3)

was also present. Narrow-leaved dwarfs (5) resembling *elliptica* appeared and there were also broad-leaved dwarf rosettes (4). Since none of these variants show any of the *brevistylis* peculiarities they have been grouped with *Lamarckiana* in calculating the proportions of *brevistylis* in the cultures.

The most important problem presented is an understanding of why the ratios of *brevistylis* to *Lamarckiana* and other plants in the cultures should present so great a range of variation in the results offered for comparison. Two possible explanations suggest themselves. (1) It is possible that sister plants of the same cross may differ in their genetical constitutions and for this reason some may throw *brevistylis* in larger or smaller proportions than others. (2) Or, it is possible that variations in the ratios of *brevistylis* may result from environmental factors, as for example malnutrition, which operate to prevent the full expression of zygotes that normally should give viable seeds of *brevistylis*.

I am working on the hypothesis that the latter explanation is more likely to account for irregularities of ratios and shall discuss the data here presented from that standpoint. It should, however, first be noted that there are sure to be uncertainties of data with material in which some plants in the cultures die generally as seedlings, frequently because they are unable to throw off their seed coats. This condition can only be fully appreciated through cultures in which the seeds are experimentally germinated and the fate and condition of all are fully recorded. Such data are obviously impossible to obtain in earth-sown cultures. This mortality must increase or diminish the ratios, as recorded, according to the nature of the dead plants whether or not *brevistylis*. Therefore, variations from expected ratios are more likely to occur than in material in which generations can be brought to maturity with little or no loss by death.

There are assembled in table 9 the cultures which presented *brevistylis* as a segregate in ratios close to expectations on the hypothesis of a monohybrid situation; and in table 10 are brought together the cultures in which the ratios of *brevistylis* were far from expectations. The full data for all of the cultures are given in tables 1-4 to which reference is made in the first column, and this data must be considered in an examination of tables 9 and 10. Thus in table 9 cultures 15.15 *d* and 17.64, and in table 10 culture 17.68, give ratios of little significance (apart from the important fact of a segregation) because the cultures were small and the mortality high. There are no indications from these records in particular that ratios from cultures in Petri dishes are less irregular

TABLE 9

Cultures presenting the segregation of brevistylis in proportions close to expectations on the hypothesis of a monohybrid situation.

Culture	Its character	Seeds sown in	Percentage of germination	Ratio of *brevistylis* to *Lamarckiana* and other plants	Ratio to be expected by the hypothesis
15.15 *b* (Table 1)	F_2 from $L \times b$	Earth	17	1 : 3.1	1 : 3
15.15 *d* (Table 1)	F_2 from $L \times b$	Earth	5.37	1 : 2.2	1 : 3 (Small culture and high mortality make data of little value)
SHULL 14177 (Table 1)	F_2 from $L \times b$	Earth	17.4	1 : 3.8	1 : 3
16.15 *g* (Table 1)	F_2 from $L \times b$	Petri dish	37.8	1 : 3.28	1 : 3 (High percentage of germination)
15.41 (Table 1)	F_2 from $L \times b$	Earth	34.3	1 : 2.2	1 : 3
15.42 (Table 1)	F_2 from $L \times b$	Earth	27.8	1 : 2.7	1 : 3
17.52 (Table 2)	F_2 from $b \times L$	Petri dish	37.1	1 : 2.6	1 : 3 (High percentage of germination)
17.54 (Table 2)	F_2 from $b \times L$	Petri dish	35.7	1 : 2.5	1 : 3 (High percentage of germination)
17.61 (Table 3)	Double reciprocal $(L \times b) \times (b \times L)$	Petri dish	29.1	1 : 4	1 : 3 (Fair percentage of germination)
17.62 (Table 3)	Double reciprocal $(b \times L) \times (L \times b)$	Petri dish	32.6	1 : 2.5	1 : 3 (Fair percentage of germination)
17.63 (Table 4)	Iterative $(L \times b) \times b$	Petri dish	25.5	1 : 1.5	1 : 1 (Fair percentage of germination)
17.64 (Table 4)	Sesquireciprocal $b \times (L \times b)$	Petri dish	32.8	1 : 1	1 : 1 (Small culture and very high mortality make data of little value)
17.67 (Table 4)	Sesquireciprocal $(b \times L) \times b$	Petri dish	35.5	1 : 1.7	1 : 1 (High percentage of germination)

than those from earth-sown cultures but of course the percentages of germination for the former have significance as accurate tests of seed sterility while the percentages for the latter may or may not be approximations to the facts of seed viability.

An examination of table 9 (with ratios of *brevistylis* close to expectations) will show that the percentages of germination in Petri dish cultures is generally high. Of the eight cultures in Petri dishes only two showed a germination below 32 percent, these being cultures 17.61 (29.1) and 17.63 (25.5) both of which present *brevistylis* in proportions lower than expectations. The other six cultures range from 32.6 percent (culture 17.62) to 37.8 percent (culture 16.15 *g*).

In table 10 with ratios of *brevistylis* far from expectations, the percentages of germination recorded for Petri dish cultures is much lower than for the cultures assembled in table 9. Excluding culture 17.68 where the ratio, as already noted, has little significance, there are five cultures to be considered. Among these the highest percentage of germination is 33.3 (culture 16.15 *j*) and the other four cultures are below 31 percent with the two lowest percentages 15.8 (culture 16.15 *i*) and 19 (culture 16.15 *h*). Of the greatest significance perhaps is the fact that *brevistylis* was in its lowest proportions to *Lamarckiana*, 1 : 9 and 1 : 9.8, in cultures 16.15 *i* and 16.15 *h* where the percentages of germination were the lowest (15.8 and 19) recorded for Petri dish cultures.

The data of tables 9 and 10 may be examined from another angle. There are only three Petri dish cultures (table 9, cultures 17.52, 17.54 and 17.62) in which *brevistylis* appeared in proportions higher than expectations and in these the ratios vary from expectations by no more than 0.5. This perhaps represents a departure no greater than might be expected in material so difficult to bring to complete expression as these Oenotheras. On the other hand all of the other nine cultures in Petri dishes (17.64 and 17.68, excluded) present *brevistylis* in lower proportions than expectations and the departures range from 0.28 (16.15 *g*) to 6.8 (16.15 *h*) with eight cultures showing a variance above 0.5. In five of these nine cultures (table 10) the variance from expected ratios ranged from 2.4 to 6.8. On the hypothesis of a monohybrid situation in these crosses the departures from expected ratios is only marked where *brevistylis* appears in fewer numbers and this condition appears to be correlated with lower percentages of germination, such as might indicate a differential mortality working against the formation of *brevistylis* zygotes or against their development.

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TABLE 10

Cultures presenting the segregation of brevistylis in proportions far from expectations on the hypothesis of a monohybrid situation.

Culture	Its character	Seeds sown in	Percentage of germination	Ratio of *brevistylis* to *Lamarckiana* and other plants	Ratio to be expected by the hypothesis
15.15 *c* (Table 1)	F_2 from $L \times b$	Earth	10.9	1 : 5.5	1 : 3
SHULL 14169 (Table 1)	F_2 from $L \times b$	Earth	31.2	1 : 6.5	1 : 3
16.15 *h* (Table 1)	F_2 from $L \times b$	Petri dish	19	1 : 9.8	1 : 3 (Low percentage of germination)
16.15 *i* (Table 1)	F_2 from $L \times b$	Petri dish	15.8	1 : 9	1 : 3 (Low percentage of germination)
16.15 *j* (Table 1)	F_2 from $L \times b$	Petri dish	33.3	1 : 5.4	1 : 3 (Fair percentage of germination)
17.51 (Table 2)	F_2 from $b \times L$	Petri dish	28.9	1 : 5.6	1 : 3 (Fair percentage of germination)
17.53 (Table 2)	F_2 from $b \times L$	Petri dish	30.0	1 : 6.2	1 : 3 (Fair percentage of germination)
17.68 (Table 4)	Iterative $b \times (b \times L)$	Petri dish	37.8	1 : 8	1 : 1 (Small culture and high mortality make data of little value)

TABLE 11

Petri dish cultures arranged in order beginning with those in which the proportions of brevistylis are far below expectations. Note the marked rise in the percentages of germination among the cultures in which the ratios of brevistylis approach expectations.

Cultures	For full data see	Recorded ratios of *brevistylis*	Ratio of expectations	Percentages of germination	Remarks
17.68	Table 10	1:8	1:1	37.8	Small numbers and high mortality make the data for culture 17.68 of little value (see table 4).
16.15 *h*	Table 10	1:9.8	1:3	19	
16.15 *i*	Table 10	1:9	1:3	15.8	
17.53	Table 10	1:6.2	1:3	30.9	
17.51	Table 10	1:5.6	1:3	28.9	
16.15 *j*	Table 10	1:5.4	1:3	33.3	
17.61	Table 9	1:4	1:3	29.1	
17.67	Table 9	1:1.7	1:1	35.5	
17.63	Table 9	1:1.5	1:1	25.5	Small numbers and very high mortality make the data for culture 17.64 of little value (see table 4).
16.15 *g*	Table 9	1:3.28	1:3	37.8	
17.64	Table 9	1:1	1:1	32.8	
17.52	Table 9	1:2.6	1:3	37.1	
17.54	Table 9	1:2.5	1:3	35.7	
17.62	Table 9	1:2.5	1:3	32.6	

Arranging the Petri dish cultures in order beginning with those in which the proportions of *brevistylis* are lowest we find that the percentages of germination at the beginning of the series are in general much lower than those recorded for the cultures in which *brevistylis* is close to expectations or in somewhat greater proportions. The list is given in table 11 and the marked rise in germination percentages among the cultures in which the ratios of *brevistylis* approach expectations is evident.

A list of earth-sown cultures similarly arranged is presented in table 12. In this table there will not be found so regular a correlation of low proportions of *brevistylis* with low percentages of germination as in the list of Petri dish cultures (table 11), but this does not seem to me surprising in view of my observations on the wide variation in the rate of germination exhibited by earth-sown cultures of Oenotheras (DAVIS 1915 *b*, p. 237; 1917, pp. 168, 177). In earth-sown cultures incomplete germination may readily result in marked departures from correct ratios according as various physiological factors, about which we know very little, may favor somewhat either *brevistylis* or *Lamarckiana*, or work against the germination of their viable seeds.

TABLE 12

Earth-sown cultures arranged in order, beginning with those in which the proportions of brevistylis are far below expectations. Note that there is not present so striking a correlation of low proportions of brevistylis with low percentages of germination as shown in table 11, a fact possibly due to irregularities and variations in the rates of germination under the physiological conditions of earth-sown cultures.

Cultures	For full data see	Recorded ratios of *brevistylis*	Ratios of expectations	Percentages of germination	Remarks
15.15 *c*	Table 10	1:15.5	1:3	10.9	
SHULL 14169	Table 10	1:6.5	1:3	31.2	
SHULL 14177	Table 9	1:3.8	1:3	17.4	Small numbers and high mortality make the data for culture 15.14 *d* of little value (see table 1).
15.15 *b*	Table 9	1:3.1	1:3	17	
15.42	Table 9	1:2.7	1:3	27.8	
15.41	Table 9	1:2.2	1:3	34.3	
15.15 *d*	Table 9	1:2.2	1:3	5.37	

This finishes the consideration of the data presented in this paper. It might be more extensive and it is unavoidably mixed in its nature since both earth-sown cultures and cultures from complete germination in Petri dishes are considered, but I trust that its presentation is justified together with the working hypothesis which is offered for the interpretation of the results. This hypothesis presupposes that the group of associated characters which distinguishes *brevistylis* from *Lamarckiana* is recessive to the characters of *Lamarckiana* and that the situation in F_1 generations from crosses of *brevistylis* with *Lamarckiana* is that of a monohybrid. The hypothesis also assumes that the segregation of *brevistylis* which appears in the F_2, would follow when fully expressed the simple Mendelian ratio of 1:3, and that the back cross of the F_1 to the recessive *brevistylis* would give the familiar ratio of 1:1 if undisturbed by extraneous factors. Proportions of *brevistylis* lower than expectations on this hypothesis are believed to be due to a differential mortality affecting the *brevistylis* segregates and expressed by lower percentages of germination in those cultures that exhibit such departures from the expected ratios.

There is an experimental method of attack on the problems developed by the data of this paper for which plans have been laid and preliminary steps taken. Plants of the F_1 generation of reciprocal crosses between *brevistylis* and *Lamarckiana* have so long a flowering season that it is

possible to self and collect a harvest of ripened seeds by the middle of August. The plants at this time in the summers of Philadelphia are still in fine flower and a second harvest of selfed seed may easily be obtained before the end of the season. Thus it is possible to experiment on selected plants, the breeding behavior of which may be established by cultures of their seeds ripened earlier in a flowering season. Certain strong plants from reciprocal F_1 crosses were chosen in the summer of 1917, selfed, and their seed ripened in the early part of the season when the parent plants were in full vegetative vigor. After harvesting the seed these same plants were subjected by the removal of branches and leaves to a treatment certain to place the selfed seeds of a second harvest under conditions of malnutrition in varying degrees of severity. Will the proportions of *brevistylis* in F_2 cultures from seeds of the second harvest (subjected to malnutrition) be found to differ markedly from the proportions in cultures of seeds ripened on the same plants early in the season under conditions of very healthy vegetative vigor?

SUMMARY

1. The F_1 generations of reciprocal crosses between *Oenothera brevistylis* and *Oe. Lamarckiana* are uniform and essentially indistinguishable from one another. The characters of *Lamarckiana* are strongly dominant and the hybrids may be separated from *Lamarckiana* only when measurements of strongly contrasted characters of the parents are compared. Then the influence of the *brevistylis* parent may appear in styles not quite so long as those of *Lamarckiana,* in leaves and bracts somewhat broader, bud cones a little thicker, and sepal tips somewhat shorter.

2. The F_2 generations from reciprocal F_1 hybrids present a perfectly sharp segregation of *brevistylis* plants from *Lamarckiana* in proportions approximating the monohybrid Mendelian ratio of 1 *brevistylis* : 3 *Lamarckiana.* Thus the distinguishing characters of *brevistylis* are inherited in closely associated relations, behaving as a unit. Marked departures from this 1 : 3 ratio are in the direction of lower proportions of *brevistylis* and appear to be correlated with smaller percentages of viable seeds, indicating that a differential mortality may work against the appearance of *brevistylis* and thus modify the Mendelian ratio of expectation (tables 1, 2, 9, 10).

3. Double reciprocal crosses, as would be expected, gave ratios of *brevistylis* to *Lamarckiana* close to 1 : 3 and here also a smaller proportion of *brevistylis* was accompanied by a lower percentage of seed germination (tables 3, 9).

4. Back crosses of the reciprocal F_1 hybrids to the parent *Lamarckiana* (table 4) gave progenies with the characters of *Lamarckiana* as would be expected from the dominance of *Lamarckiana* shown in the F_1 generations of crosses between the parent species.

5. Back crosses of the reciprocal F_1 hybrids to the parent *brevistylis* present the same clear segregation of *brevistylis* exhibited by the F_2 generations and by the double reciprocal crosses. The proportions of *brevistylis* to *Lamarckiana* approximated the ratio of 1 : 1 to be expected when a monohybrid is crossed with its recessive parent stock (tables 4, 9).

6. Variants appeared in the cultures from seeds forced to complete germination but were not noted in earth-sown cultures. Some of these were clearly the "mutants," *albida, nanella, lata, rubrinervis,* and narrow-leaved dwarfs close to *elliptica.* Others were similar to *oblonga* and *laevifolia.* Certain broad-leaved dwarf rosettes were also present.

7. *Oenothera brevistylis* breeds true in agreement with its evidently recessive nature.

8. A selfed line of *brevistylis* has been started, the plants of which show a wide range in the length of style, perfection in the development of stigma lobes, and yield of seed. Selection towards a more perfect expression of these organs and a higher yield of seed has been practiced for two generations. The results so far have not led to improvement.

9. The breeding data indicate that crosses between *brevistylis* and *Lamarckiana* result in a simple monohybrid situation. On this assumption the hypothesis is proposed that the variations in the expected ratios of *brevistylis* as a segregate in the F_2 generations, double reciprocal crosses, and back crosses of the F_1 to *brevistylis* result from environmental factors, as perhaps malnutrition, which operate to prevent the expression of zygotes that normally should give viable seeds of *brevistylis.* Such factors if operative should on the hypothesis lead to lower percentages of viable seeds, and apparently lower percentages of viable seeds are correlated with lower proportions of *brevistylis* as a segregate. Steps have been taken to test this hypothesis by an experimental method of attack.

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HERITABLE EFFECTS OF TEMPERATURE DIFFERENCES ON THE FISSION RATE OF *STYLONYCHIA PUSTULATA*[1]

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[1] From the Zoölogical Laboratory of the JOHNS HOPKINS UNIVERSITY.

INTRODUCTION

The present paper is a contribution to the study of the question of how living systems may become modified so that the modifications remain in later generations, even after the modifying factors are removed. The living system shows a great power of compensating for disturbances induced in it; if the compensation does not come in the same generation that is affected, it usually comes in the next one, so that "acquired characters are not inherited." Can we change the living system so that the change will persist into later generations?

To attack this question, the simplest possible conditions are here selected. The organism is an infusorian multiplying by simple fission. The character selected is one most easily modified, namely, the rate of fission. This can be changed by all sorts of conditions; but a given fission rate is, under given conditions, characteristic for a given stock; it is thus heritable in this kind of reproduction. As a modifying agent temperature was chosen. The experiments are therefore most simple in plan. Half the progeny of a given individual are subjected to high temperature, half to low temperature. The former divide rapidly, the latter slowly. They are kept for many generations under these diverse conditions. Finally, we bring both sets again to the same temperature and allow them to multiply. Will any effect have been produced, such as to show in the later history of the race? Or will both sets compensate at once their different fission rates, and return to the rate that they would have shown had they not lived under different conditions? If a lasting modification has been produced, will it be in the same direction as the immediate action of the external agent, so that those that have been in the high temperature continue to divide more rapidly at the intermediate temperatures? Or will there possibly be a swing in the opposite direction, so that those that have been dividing slowly, in the low temperatures, now divide more rapidly in the intermediate temperature?

So far as I have been able to discover, no studies have been made to test these matters. Many investigations have been made of the immediate effect of diverse temperatures on the rate of fission, such as those of MAUPAS (1888, 1889), CALKINS (1902), WOODRUFF and BAITSELL (1911) and others. But none of these studies touches the specific problem here investigated.

The organism employed, *Stylonychia pustulata,* is the same one used in my former study, on the effects of selection on the fission rate (MIDDLETON 1915).

In the experiment here described, tests of the lasting effect of divers-

ity of temperature were made under many conditions; when the diversity of conditions had lasted but a short time, and when it had acted for moderate and for long periods. Further, sometimes, the tests were made with both sets under a common intermediate temperature; sometimes with both under the same high temperature, sometimes with both under a low temperature.

Three long series of experiments were carried on. In one of these the two halves of a single stock were kept for eight months under diverse temperatures, with tests of their progeny at various intervals and under various conditions. The other two series were shorter; one was a preliminary series; the other followed the main series. Diagrams of the course of the three series are given in figure 1 (page 538), figure 3 (page 546), and figure 5 (page 564).

The work was undertaken in the Zoölogical Laboratory of the JOHNS HOPKINS UNIVERSITY, and I am gratefully indebted to Professor H. S. JENNINGS for valuable criticisms.

METHODS

In order that results of the study of the differential effects of a particular environmental agent on a character so delicately responsive to all sorts of agents as the fission rate of an infusorian may be of value, other conditions must be kept uniform. To secure this uniformity and to guard against contamination of the lines by exchange of individuals, or by introduction of an outside individual, a modification of the method described by JENNINGS (1913) was used.

As culture medium, 1/16 percent Horlick's malted milk was employed, prepared as described in my previous paper (MIDDLETON 1915, p. 455). The animals were cultivated on ground-glass slides, each bearing two circular depressions capable of holding four or five drops of liquid. These were kept in moist chambers. Three drops of culture medium were used in each depression, and no cover glasses were employed. Half the slides were subjected to higher temperatures (giving the "high lines"), half to lower temperatures (giving the "low lines"). The word "line" is here used as a convenient designation for the series of individuals derived in a given experiment from a single parent. All the progeny of a single parent through fission are designated a *clone*.

Uniformity of bacterial content in the culture medium was secured by washing the animals in fresh culture medium before every transfer to a new slide. The animals were allowed to swim about for a time in the fresh medium, in order to wash themselves largely free of bacteria;

they were then transferred to the definitive slide in new fluid. The pipette used in transferring the individuals was invariably sterilized in boiling water after each transfer, thus preventing the unintentional introduction of any individual which might cling to the pipette; there was thus no possibility of admixture of lines. The slides were labelled in lead pencil; the number of fissions and selections at each examination were likewise recorded on them, to be later transferred to permanent records. The individual lines were designated in accordance with the plan set forth by JENNINGS (1913).

Each concavity contained characteristically one individual. The slides were examined every other day or oftener, and all but one of the individuals that had been produced were removed. Thus the number of lines and of individuals was kept constant. The remaining individual was transferred to a new slide, with fresh fluid. In deciding which individual should be thus retained to carry on the line, "balanced selection" was practiced throughout, in accordance with the plan set forth in my former paper (MIDDLETON 1915, p. 459). This is based on the following considerations. Often one of the two individuals formed by the division of a given parent divides before the other. In my earlier paper I showed that by continued selection, on the one hand of the individuals that divide early, on the other hand of those that divide late, two sets are finally produced with characteristically diverse fission rates. For our present purposes it is necessary to avoid this effect of selection. This is done by choosing on alternate days first the faster, then the slower individual, if any difference exists. The effect of any selection toward rapid fission is thus compensated at the next transfer by a reverse selection toward slow fission. (For details see my previous paper.)

In drawing off progeny from each of the two sets of lines ("high" and "low") for tests under intermediate temperatures, the original stocks were still kept under the diverse temperatures. That is, when test lines are to be started, half the individuals of a given line are left under the old temperature, half placed under the new intermediate temperature. Each line is thus doubled, its two halves serving as controls one for the other, as to the effects of the new conditions.

FIRST SERIES OF EXPERIMENTS

(1) *General outline*

The first series of experiments was a preliminary one, to feel out the problem and to determine the best methods of procedure. The plan of

the experiments was not so well worked out, nor the conditions so accurately controlled as in later experiments.

The course of the series, with the conditions under which the organisms were kept, the periods of time, and the various tests made, are indicated in figure 1. The plan was in brief as follows:

One hundred and twenty animals of the tenth generation from a single individual of *Stylonychia pustulata,* from a 'wild' laboratory culture, were isolated on the slides, one animal in each concavity (on June 17, 1915). Sixty of the 120 lines so formed constituted the "high" set, being kept at a temperature of 30° to 32° centigrade; the other sixty formed the "low" set, being kept at 18° to 20° centigrade. They were thus propagated for twenty days, records being kept of the numbers of fissions (A, figure 1). At the end of twenty days, the first test was made; progeny from both sets were placed at the room temperatures (B, figure 1). Meanwhile, the two original sets were allowed to propa-

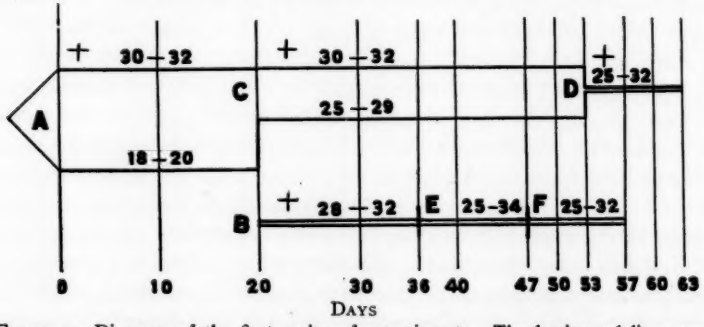


FIGURE 1.—Diagram of the first series of experiments. The horizontal lines represent the cultures, the vertical lines show the number of days through which the cultures are carried. The numbers above the horizontal lines show the temperatures (centigrade) at which the cultures were kept. The upper lines show the basic cultures with their direct continuations. The double lines below are the parallel test cultures, derived from the basic cultures and kept in progress at the same time, but with both sets under the same temperature. The beginning, duration and end of each culture or test are shown. A + sign above the test shows that the high-temperature set divides more rapidly; below, it shows that the low-temperature set divides more rapidly.

A. Two sets of 60 lines each derived from the same parents, kept for 20 days at diverse temperatures; the high-temperature set divide more rapidly. B. The first test, both sets at room temperature for 16 days; the ex-high set continues to divide the more rapidly. C. The basic cultures continued for 33 days as mass cultures at slightly differing temperatures. D. The second test at room temperatures; the ex-high set continues to divide the more rapidly. E. Continuation of the two cultures of the first test as mass cultures at the same room temperature. F. Third test, both sets on slides at room temperature for 10 days; no marked difference in fission rate.

gate in mass cultures for 33 days, at only slightly differing temperatures (C, figure 1)—the high set at 30° to 32°, the low set at 25° to 29°. Now the second test was made, both sets were placed on slides at room temperature for 10 days (D, figure 1), and records were kept of their rate of fission. A third test was taken from the descendants of the two sets of test 1. These at the end of test 1 were kept for eleven days in mass cultures at room temperatures (E, figure 1), then placed for ten days on slides at room temperatures, and records kept of their fission rates (F, figure 1).

In this series therefore we have to deal with (1) the direct effect of the diverse temperatures on fission rate during the first twenty days (A, figure 1); (2) with the fission rates in the three tests (B, D, F, figure 1) in which the members of the two sets were kept at the same temperature, after various periods in diverse temperatures.

(2) *The direct effect of the diverse temperatures on the fission rate (A, figure 1)*

Table 1 gives the actual numbers of fissions for each set for the two ten-day periods during which they were subjected to the diverse temperatures. Each set contained sixty lines; in order to make it possible to judge of the uniformity of the effects, each set is divided into six groups of ten lines each; a given group of the high set is compared with the similarly numbered group of the low set. This grouping is based entirely on the numbering of the lines at the beginning, so that it is purely random.

TABLE 1

SERIES I.—*Number of fissions (generations) per line for the two sets of sixty lines each, of the first clone, during the period A of figure 1, the high set kept at 30° to 32° C., the low set at 18° to 20° C., with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number						Total 60 lines	Average per line per 10-day period	Percent that the difference is of the total in all
	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60			
First 10-day period									
30° to 32° C. high	299	191	237	168	163	215	1273	21.21	
18° to 20° C. low	198	176	218	202	227	225	1246	20.76	
Excess of high	101	15	19	—34	—64	—10	27	.45	1.07
Second 10-day period									
30° to 32° C. high	378	369	308	291	161	304	1811	30.18	
18° to 20° C. low	139	141	161	155	165	142	903	15.05	
Excess of high	239	228	147	136	—4	162	908	15.13	33.47

As table 1 shows, during the first ten days the animals kept at the higher temperature divided little more rapidly than those kept at the lower temperature; indeed, in three of the six groups of each set those kept at the lower temperature divided more rapidly than the corresponding group kept at the higher temperature, and the average for the two sets was almost the same. But in the second ten days the set kept at the higher temperature divided on the average twice as rapidly as that kept at the lower temperature. The "high" group averaged 3.018 fissions per day for each line, the "low" group but 1.505 fissions per day. In the first half of figure 2 is plotted the difference in number of fissions between the two sets for the successive two-day periods of the experiment. In this figure the straight base line or abscissa represents the number of fissions for each two days, in the sixty lines that were kept at low temperature, while the irregular line shows the number of fissions above or below this, produced in the same period by the 60 lines kept at high temperature. Throughout the second ten days the set at high temperature are consistently above the others, the excess increasing rapidly toward the end of the twenty-day period. The difference in temperature seems to have a cumulative effect.

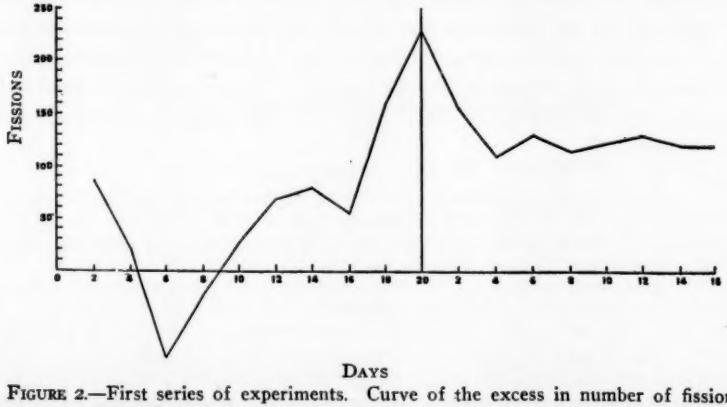


FIGURE 2.—First series of experiments. Curve of the excess in number of fissions in the set cultivated at high temperature over the number in the set cultivated at low temperatures. The first part of the curve to the vertical line at 20 days, shows the excess while the two sets were in the different temperatures, the high-temperature set cultivated at 30 to 32 degrees, the low set at 18 to 20 degrees (A, figure 1). The second half, to the right of the vertical line, shows the excess of the ex-high set when both sets are cultivated at the same room temperature (B, figure 1). The curve is plotted for two-day periods, the horizontal scale showing the number of days, the vertical scale the excess in fissions of the high-temperature set over the low-temperature set.

(3) *First test of the permanence of modification* (*B, figure 1*)

Now the two sets of sixty lines each, one of which has lived for twenty days at 30° to 32°, the other for the same period at 18° to 20°, are both brought to room temperature. Will they continue to show a difference in fission rate? And, if so, of what nature will be the difference?

The period of this test was 16 days, from July 7 to July 22, inclusive. The two sets were kept at the same room temperature, but at this period of the year the temperature is high in Baltimore, and it gradually rose throughout the period of test. The records show that for each of the first four days the maximum temperature reached was 28.5 degrees; in each of the next four it was 30 degrees; in each of the next four, 32 degrees, and on the fourteenth day it reached its highest point, at 32.5 degrees. The minimum temperatures for the various days varied from 25 degrees to 29 degrees.

Thus the temperature at which both sets were now kept was much nearer that at which those cultivated under high temperature had been kept than to that at which the "low" set had been kept. In other words, there was now relatively little change of temperature for the "high" set, while the "low" set are raised to a much higher temperature than that to which they have hitherto been accustomed. But the two sets were now at all times under the same temperature.

Table 2 gives the number of fissions for each set of lines, by groups of ten, during these 16 days of subjection to the same temperature, with the excess in favor of the set that had previously been kept under the high temperature. This set continues throughout the period to maintain its higher rate of fission, falling only slightly from the average rate of 3.018 fissions per day for the 10 days preceding this test to 2.889 per day for the 16 days of the test. The set which had been kept in low temperature increases its fission rate on being placed at the higher temperature of the test, but not so much as to reach that of the other set. During the last 10 days of low temperature, its fission rate had averaged for each line 1.505 per day; during the 16 days of the test, it rises to an average of 1.844 fissions per day. The second half of figure 2 gives the curve of the difference in number of fissions by two-day periods, while the two sets were at the same temperature. The ex-high set remains consistently above the ex-low set.

Thus in this first test it appears that a heritable difference has been produced of the same nature as the direct difference produced by the diversity of temperature. The ex-high set retains its higher rate; the

TABLE 2

SERIES I.—*First test (B, figure 1). Number of fissions (generations) per group of ten lines for the two sets of sixty lines each, both sets now kept at the same temperature (after twenty days at diverse temperatures), with the average number of fissions for each line, and the excess in favor of the ex-high lines.*

	Lines number						Total	Average per line for the whole period	Average per line per day	Percent that the difference is of the total in all
	1 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60				
First 10-day period										
Ex-high lines.....	298	287	302	342	264	299	1792	29.86	2.986	
Ex-low lines.....	187	185	195	196	204	192	1159	19.31	1.931	
Excess of ex-high....	111	102	107	146	60	107	633	10.55	1.055	21.46
Following 6-day period										
Ex-high lines.....	156	150	164	199	131	181	981	16.35	2.725	
Ex-low lines.....	106	106	109	107	98	90	616	10.27	1.712	
Excess of ex-high....	50	44	55	92	33	91	365	6.08	1.013	22.83

ex-low in a measure also its lower rate, impressed upon them by the previous differences in temperature.

(4) *Second test of the permanence of modification* (*D, figure 1*)

After the first twenty days on slides in diverse temperatures, parts of the lines not employed in the first test were transferred, unwashed, to small mass cultures, the high temperature lines forming one culture, the low temperature lines another. The culture fluid was .031 percent malted milk. The high-temperature mass culture was placed in an incubator kept at the same temperature as the high-temperature slides, i.e., at 30 to 32 degrees centigrade. The low-temperature culture was kept in an incubator at 25 to 29 degrees centigrade. The two were allowed to multiply at these slightly differing temperatures in the mass cultures for 33 days (see C, figure 1). Thus the two sets had been kept at diverse temperatures for 53 successive days, since the beginning of the experiment.

Now, on August 2, thirty individuals from each of the cultures were isolated on slides and allowed to multiply for 10 days at room temperature, records being kept of their rate of fission (D, figure 1). The room temperature varied during this period from 25 to 32.5 degrees, so that again the common temperature at which the two sets were kept was high. Table 3 gives the number of fissions during the ten days for each of the thirty lines of the two sets, with comparison between the two lines designated by the same number in the two sets. (These designations are of course given at random, before the records are taken.)

It will be observed that every member of the ex-high set showed a greater number of fissions than any member of the ex-low set. On the average, each ex-high line produced during the ten days 6.37 more generations than each ex-low line. Here again therefore, it appears that the earlier differences in temperature have produced a modification of the fission rate that lasts after the diversities in temperature have disappeared, and this lasting modification is in the same direction as the immediate effect produced by the diverse temperature.

(5) *Third test of the permanence of modification* (*F, figure 1*)

At the close of the first test (B, figure 1), the two halves of the clone had been first kept for 20 days at diverse temperatures, then for 16 days at the same temperatures; during these 16 days we had found that the ex-high set continued to multiply more rapidly than the ex-low set. To

TABLE 3

SERIES I.—*Number of fissions per line for the two sets (ex-high and ex-low), in the second test of 10 days under the same temperature (D, figure 1). Thirty lines in each set. The table gives the number of fissions for each line during the ten days of the test, with comparison between the like-numbered lines of each set, together with the excess in favor of the ex-high lines. The room temperature at which both were kept ranged from 25 to 32.5 degrees.*

	Lines number																													Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
Ex-high	33	32	33	33	34	34	32	29	34	32	34	34	34	32	34	32	36	36	31	34	33	32	32	36	34	32	32	35	34	35	998	33.27	
Ex-low	29	29	27	25	25	27	28	28	25	27	23	28	24	28	22	26	26	26	29	27	23	29	29	31	27	27	28	27	29	28	807	26.90	
Excess of ex-high...	4	4	6	8	9	7	4	1	9	5	11	6	10	4	12	6	10	10	2	7	10	3	3	5	7	5	4	8	5	7	191	6.37	10.58

TABLE 4

SERIES I.—*Number of fissions per line for the two sets (ex-high and ex-low), in the third test of 10 days under the same temperature (F, figure 1). Thirty lines in each set; both kept at room temperature (25 to 32.5 degrees). The previous history of the two sets can be seen in figure 1. The table gives the number of fissions for each line, with comparison between the like-numbered lines of each set, together with the excess in favor of the ex-high lines. The minus sign indicates an excess in favor of the ex-low lines.*

	Lines number																													Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
Ex-high	23	22	23	21	26	26	27	29	23	27	27	26	28	25	28	26	24	26	26	24	24	22	26	25	26	26	25	23	29	27	760	25.33	
Ex-low	28	28	24	25	28	29	28	30	31	28	30	28	28	30	30	30	29	29	28	28	27	28	30	31	30	28	29	27	26	27	852	28.40	
Excess of ex-high...	−5	−6	−1	−4	−2	−3	−1	−1	−8	−1	−3	−2	0	−5	−2	−4	−5	−3	−2	−4	−3	−6	−4	−6	−4	−2	−4	−4	3	0	−92	−3.07	−5.71

see whether this difference would continue, the two sets were converted into two small mass cultures which were kept at room temperature (25 to 34.5 degrees) for 11 days (E, figure 1), then the fission rate of the two was again compared, by cultivation on slides for ten days at room temperature (25 to 32.5 degrees). Thirty individuals were isolated from each of the two sets, numbered at random, and cultivated on slides under identical conditions (F, figure 1). Table 4 gives the actual number of fissions for each of the thirty lines of each set during the ten days of the test.

Table 4 shows that the ex-high lines no longer divide more rapidly than the ex-low lines, indeed, in most cases the ex-low lines divide more rapidly. In 26 out of the 30 pairs made by comparing lines of like designation, the ex-low lines show more divisions than the ex-high lines. The average excess per line for the ten days is 3.07 fissions in favor of the ex-low lines.

Thus the higher fission rate produced by the earlier high temperature has disappeared; it is possibly even replaced by a lower fission rate, although the difference is small and perhaps does not justify drawing conclusions on this point.

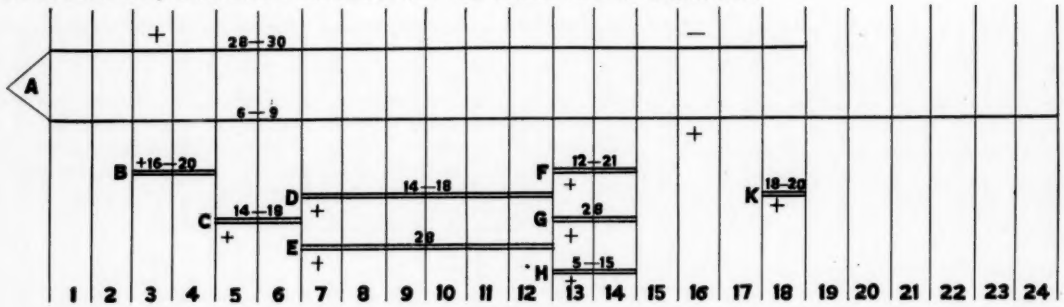
(6) *General results of the first series of experiments*

The experiments of this first series tend to show that diversities of temperature produce a diversity of fission rate which lasts after the diversity of temperature has ceased. In two tests made immediately after the subjection to diversity of temperature, the lasting difference in fission rate was of the same character as the direct effect produced by the different temperatures; the lines that had been subjected to the higher temperature continue to divide more rapidly after the two sets are returned to the same temperature, and this difference lasts as much as 16 days. But in a test at common temperature made 37 days after the lines had been returned to the common temperature, the difference in favor of the ex-high lines was no longer in evidence; it was indeed replaced by a slight excess in fission rate in favor of the ex-low lines.

SECOND SERIES OF EXPERIMENTS

(7) *General outline*

The results of the preliminary series of experiments, described above, appeared to justify a careful study of the phenomena, on a larger scale, and with more precise control of the conditions. Accordingly, on Oc-



TEN-DAY PERIODS

FIGURE 3.—Diagram of the extensive second series of experiments. The horizontal lines show the duration of the cultures, the vertical lines showing the number of 10-day periods through which they are carried. The numbers above horizontal lines show the temperatures at which the cultures were kept.

The two upper horizontal lines show the basic cultures, kept at diverse temperatures. The double lines below are the parallel test cultures, derived from the basic cultures and kept in progress at the same time, but with both sets of cultures under the same temperature. The beginning, duration and end of each culture or test are shown. A + sign above the test lines shows that the high temperature set (or the ex-high-temperature set) divides the more rapidly; a + sign below the test lines shows that the low-temperature set (or ex-low-temperature set) divides the more rapidly.

A. Two sets of 30 lines each derived from a single parent and kept at diverse temperatures for eight months. The high temperature set divides more rapidly at first, but decreases after about four months; and after 150 days its rate falls below that of the low-temperature set; all its members are dead at the end of 171 days. The low-temperature set is discontinued at the end of 240 days.

B. First test after 20 days; the ex-high set divide more rapidly.

C. Second test, after 40 days; the ex-low set divide more rapidly.

D to K. Tests at the diverse periods and temperatures shown; in every case the ex-low-temperature set divide more rapidly.

tober 4, 1915, an extensive series of experiments was set on foot, using the progeny of a single individual taken from a 'wild' laboratory culture of *Stylonychia pustulata*.

A diagram of this second series of experiments is shown in figure 3; the reader will find it well to keep this diagram before him in following the experiments. The general course of the series was as follows: The basic cultures consisted of two sets of thirty lines each, both derived from the same parent, kept at diverse temperatures for 240 days, or eight months (figure 3, A). One set ("high") was kept at a temperature of 28 to 30 degrees centigrade, the other set ("low") was kept at 6 to 9 degrees centigrade. The difference in temperature was thus much greater than that employed in the earlier series, and the temperatures were kept more constant. The number of fissions for each set was recorded throughout these eight months. (It may be noted that at the end of four months the last 10 lines of each set were discarded, so as to leave but twenty lines in each set, in place of thirty.)

At intervals, tests were made as to the permanency of any modifications induced, by taking progeny from each set and keeping the two at the same temperature. Tests were made at intermediate temperatures after subjection to diverse temperatures for 20 days (B, figure 3); again after 40 days (C); after 60 days (D and E); after 120 days (F, G. and H), and after 170 days (K, figure 3). Further, at the 60-day period, besides the test at intermediate temperatures there was a test with both sets of lines at high temperature, and at the 120-day period there were tests with both sets at low temperatures, both at intermediate temperatures, and both at high temperatures. There is of course no difficulty in having several parallel tests in progress at once, since the number of progeny produced within a few days is sufficient to set on foot many lines. All these tests are clearly indicated, with their relation to the basic cultures, in figure 3.

(8) *Results of keeping the basic stocks for eight months under diverse temperatures (A, figure 3)*

Of the sixty lines derived from the single parent individual, thirty were placed on October 8, 1915, in a refrigerator regulated to 6 to 9 degrees centigrade, the other thirty in an electric self-regulating incubator regulated to 28 to 30 degrees centigrade. The two were cultivated at these temperatures, but under otherwise similar treatment and conditions, for 240 days. The temperatures at times varied somewhat from the limits above mentioned, as shown in table 5.

TABLE 5

SERIES II.—*Basic cultures (A, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number																												Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
28°-30° C. high	24	20	20	21	23	29	26	28	28	26	26	30	29	17	24	31	23	20	21	19	21	19	20	21	19	29	20	20	17	28	699	23.30	
6°- 9° C. low	8	8	10	9	8	7	6	8	8	7	7	8	8	10	7	6	10	8	9	5	8	9	8	6	8	7	7	8	9	7	234	7.80	
Excess of high	16	12	10	12	15	22	20	20	20	19	19	22	21	7	17	25	13	12	12	14	13	10	12	15	11	22	13	12	8	21	465	15.50	49.84
Second 10-day period																																	
28°-30° C. high	29	30	30	26	35	36	37	35	32	30	27	32	32	30	36	31	29	31	29	30	28	28	28	25	26	30	28	28	30	33	911	30.37	
8°-10° C. low	2	4	6	4	5	4	5	5	5	6	3	3	3	4	4	3	3	3	3	2	2	3	2	3	3	3	4	4	3	4	108	3.60	
Excess of high	27	26	24	22	30	32	32	30	27	24	24	29	29	26	32	28	26	28	26	28	26	25	26	22	23	27	24	24	27	29	803	26.77	78.80
Third 10-day period																																	
28°-29° C. high	22	23	22	28	35	33	34	27	20	24	20	21	23	24	24	21	23	24	26	23	26	27	26	24	24	25	23	25	20	23	740	24.67	
8°-10° C. low	5	4	4	3	3	4	4	4	4	4	4	3	4	4	3	2	3	4	4	5	3	2	2	3	2	3	1	1	3	2	98	3.27	
Excess of high	17	19	18	25	32	29	30	23	16	20	16	18	19	20	21	19	20	20	22	18	23	25	24	21	22	22	22	24	17	20	642	27.40	76.60
Fourth 10-day period																																	
26°-30° C. high	23	29	27	29	31	37	32	27	30	30	25	29	30	30	28	27	29	28	29	31	29	29	30	28	30	27	27	30	26	30	867	28.90	
6°- 8° C. low	1	1	2	1	3	3	1	2	2	3	1	1	1	1	1	1	1	2	2	1	1	1	0	1	0	1	1	1	1	1	39	1.30	
Excess of high	22	28	25	28	28	34	31	25	28	27	24	28	29	29	27	26	28	26	27	30	28	28	30	27	30	26	26	29	25	29	828	27.60	91.39
Fifth 10-day period																																	
24°-30° C. high	33	35	32	35	33	37	29	33	30	32	29	32	31	31	30	25	29	31	33	34	30	31	28	35	34	37	30	33	30	31	953	31.76	
6°-10° C. low	2	2	0	2	1	2	3	2	2	2	2	2	2	2	0	0	1	2	1	1	1	0	1	1	1	0	2	1	1	1	40	1.33	
Excess of high	31	33	32	33	32	35	26	31	28	30	27	30	29	29	30	25	28	29	32	33	29	31	27	34	33	37	28	32	29	30	913	30.43	91.96
Sixth 10-day period																																	
24°-28° C. high	19	22	19	21	18	18	15	18	20	22	16	15	17	16	20	15	16	18	18	17	21	22	19	22	21	18	19	22	20	20	564	18.80	
6°-11° C. low	2	2	1	1	1	1	2	2	3	1	3	1	2	3	3	3	3	2	2	1	0	3	2	2	2	2	3	2	1	2	58	1.93	
Excess of high	17	20	18	20	17	17	13	16	17	21	13	14	15	13	17	12	13	16	16	16	21	19	17	20	19	16	16	20	19	18	506	16.87	81.36

TABLE 5 (continued)

SERIES II.—*Basic cultures (A, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number																												Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
Seventh 10-day period																																	
24°-26° C. high	24	20	22	20	20	23	22	20	20	26	22	18	18	18	21	21	22	20	23	26	21	20	21	21	22	20	26	24	25	24	650	21.66	
6°-10° C. low	1	1	1	1	0	0	4	3	5	0	0	2	1	4	3	1	2	5	3	2	2	2	1	2	4	2	2	2	4	3	63	2.10	
Excess of high	23	19	21	19	20	23	18	17	15	26	22	16	17	14	18	20	20	15	20	24	19	18	20	19	18	18	24	22	21	21	587	19.56	82.32
Eighth 10-day period																																	
26°-28° C. high	16	14	16	11	19	21	18	15	24	24	18	19	14	14	13	12	15	12	10	11	12	12	14	12	13	13	24	20	17	19	472	15.73	
6°-15° C. low	1	1	2	1	2	2	2	2	3	1	2	2	3	1	2	3	5	4	2	1	2	4	4	2	2	3	4	2	1	3	69	2.30	
Excess of high	15	13	14	10	17	19	16	13	21	23	16	17	11	13	11	9	10	8	8	10	10	8	10	10	11	10	20	18	16	16	403	13.43	74.48
Ninth 10-day period																																	
24°-26° C. high	21	25	20	23	29	30	30	25	23	23	24	28	30	29	27	21	23	25	24	25	21	22	24	17	25	19	14	21	19	25	712	23.73	
6°-10° C. low	1	2	1	3	0	0	1	3	0	0	1	0	2	1	1	0	0	2	1	0	0	1	0	1	1	0	0	0	0	1	23	.76	
Excess of high	20	23	19	20	29	30	29	22	23	23	23	28	28	28	26	21	23	23	23	25	21	21	24	16	24	19	14	21	19	24	689	22.97	93.74
Tenth 10-day period																																	
23°-28° C. high	8	13	13	12	13	18	14	20	19	18	21	24	13	23	23	15	12	8	9	10	22	22	23	22	12	12	23	24	16	16	498	16.60	
5°-11° C. low	2	1	3	2	0	0	3	1	2	2	1	3	0	1	2	1	3	1	2	3	2	2	0	2	0	0	2	3	0	1	45	1.50	
Excess of high	6	12	10	10	13	18	11	19	17	16	20	21	13	22	21	14	9	7	7	7	20	20	23	20	12	12	21	21	16	15	453	15.10	83.42
Eleventh 10-day period																																	
24°-28° C. high	16	16	12	18	17	19	23	25	26	19	27	26	27	18	25	25	22	26	18	19	26	24	23	24	24	24	32	29	24	23	677	22.56	
4.5°-14° C. low	2	4	3	2	2	3	1	0	2	4	0	2	3	2	2	2	1	3	2	2	1	2	2	2	2	2	4	3	2	3	65	2.16	
Excess of high	14	12	9	16	15	16	22	25	24	15	27	24	24	16	23	23	21	23	16	17	25	22	21	22	22	22	28	26	22	20	612	20.40	82.52
Twelfth 10-day period																																	
23°-29.5° C. high	24	24	27	30	27	31	29	31	28	29	27	26	32	31	34	34	27	31	38	33	24	29	20	25	30	28	35	34	27	32	877	29.23	
6°-13° C. low	4	4	1	2	3	2	3	2	3	3	3	3	2	3	2	3	5	3	3	3	3	4	4	3	3	1	2	1	4	5	87	2.90	
Excess of high	20	20	26	28	24	29	26	29	25	26	24	23	30	28	32	31	22	28	35	30	21	25	16	22	27	27	33	33	23	27	790	26.33	81.95

TABLE 5 (continued)

SERIES II.—*Basic cultures (A, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Thirteenth 10-day period																							
27°-29.5° C. high	11	10	8	7	21	18	22	23	16	13	10	10	15	14	9	15	15	18	12	16	283	14.15	
5°-11.5° C. low	5	5	1	1	2	2	2	3	4	4	4	3	4	3	4	4	5	5	3	3	67	3.35	
Excess of high.....	6	5	7	6	19	16	20	20	12	9	6	7	11	11	5	11	10	13	9	13	216	10.80	61.71
Fourteenth 10-day period																							
27°-28° C. high	4	7	6	10	9	6	9	11	10	10	8	8	7	8	8	20	13	22	11	7	194	9.70	
7°-15° C. low	3	3	1	2	2	3	5	4	5	2	4	5	3	5	2	5	3	4	3	5	69	3.25	
Excess of high.....	1	4	5	8	7	3	4	7	5	8	4	3	4	3	6	15	10	18	8	2	125	6.45	49.81
Fifteenth 10-day period																							
27°-28° C. high	7	8	9	5	8	6	7	7	4	3	3	5	5	4	8	3	5	5	5	5	112	5.60	
7°-11° C. low	3	3	1	2	2	3	3	3	2	3	4	3	4	3	2	3	3	4	4	4	59	2.95	
Excess of high.....	4	5	8	3	6	3	4	4	2	0	−1	2	1	1	6	0	2	1	1	1	53	2.65	30.99
Sixteenth 10-day period																							
27°-28° C. high	5	5	6	8	5	7	5	7	10	5	1	3	3	6	2	2	4	4	4	5	97	4.85	
8°-14° C. low	3	6	6	6	5	5	5	6	7	8	6	7	6	6	7	6	6	6	6	6	119	5.95	
Excess of high.....	2	−1	0	2	0	2	0	1	3	−3	−5	−4	−3	0	−5	−4	−2	−2	−2	−1	−22	−1.10	−10.18
Seventeenth 10-day period																							
27°-28° C. high	3	3	5	6	3	8	8	5	6	8	8	7	3	3	4	3	2	3	3	3	94	4.70	
8°-14° C. low	4	6	5	5	7	8	8	7	8	7	7	7	8	7	5	7	6	7	7	7	133	6.65	
Excess of high.....	−1	−3	0	1	−4	0	0	−2	−2	1	1	0	−5	−4	−1	−4	−4	−4	−4	−4	−39	−1.95	−17.18

TABLE 5 (continued)

SERIES II.—*Basic cultures (A, figure 3). Number of fissions (generations) for the two sets of thirty lines each, of the second clone; one set kept in high temperature, the other set in low temperature; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
Eighteenth 10-day period																							
27°-28° C. high	1	3	3	3	1	1	2	2	2	0	1	0	2	1	0	0	0	0	0	0	22	1.10	
8°-14° C. low	5	5	8	7	7	6	7	6	5	5	6	7	7	5	6	5	7	7	6	7	124	6.20	
Excess of high	−4	−2	−5	−4	−6	−5	−5	−4	−3	−5	−5	−7	−5	−4	−6	−5	−7	−7	−6	−7	−102	−5.10	−69.86
Total, 180 days																							
Ex-high	290	307	297	313	347	378	362	359	348	342	313	333	331	317	336	321	309	326	313	314	6556	327.8	
Ex-low	53	62	56	54	53	55	65	63	70	62	58	62	63	65	56	55	67	72	63	58	1212	60.6	
Excess of ex-high	237	245	241	259	294	323	297	296	278	280	255	271	268	252	280	267	242	254	250	256	5344	26.72	68.80
Nineteenth 10-day period																							
6°-10.5° C. low	1	2	2	2	2	2	2	2	3	3	2	1	2	3	2	1	2	3	2	2	41	2.05	
Twentieth 10-day period																							
9°-11.5° C. low	3	3	3	2	3	2	2	3	4	2	2	3	6	6	1	0	5	6	2	2	60	3.00	
Twenty-first 10-day period																							
8.5°-17° C. low	5	5	3	2	5	2	3	3	3	1	5	8	7	9	1	2	9	9	8	2	92	4.60	
Twenty-second 10-day period																							
10°-15° C. low	4	6	4	2	5	4	4	4	1	0	5	5	4	5	4	5	6	6	5	4	83	4.15	
Twenty-third 10-day period																							
8°-14° C. low	8	9	8	8	7	8	8	9	8	8	9	9	9	10	8	8	10	10	10	9	173	8.65	
Twenty-fourth 10-day period																							
90°-13° C. low	7	7	4	6	6	6	6	6	9	6	4	5	9	7	6	8	6	9	5	9	131	6.55	
Total, 240 days																							
low	81	94	80	76	81	79	90	90	98	82	85	93	100	105	78	79	105	115	95	86		89.60	

The number of fissions for each line have been grouped by the successive ten-day periods, and are given in table 5. As in previous tables, the two lines of each set that had been designated by the same number are compared in each case. The table gives for each case also the excess in favor of the lines at high temperature; the totals for each set in each ten-day period, and the average number of fissions for each ten days for the lines of each set.

Examination of the table shows that at first the set kept at the higher temperature divided much more rapidly than that kept at the lower temperature, but that in the later periods the fission rate of the set kept at high temperatures fell off, until in the later periods the set kept at low temperature divided the more rapidly; and that finally the set kept at high temperature all died out, while the low-temperature set continued to multiply strongly. This course of the experiment is well shown in figure 4, in which the average number of fissions for each 10-day period is plotted for both sets, the continuous line showing the high-temperature set, the broken line the low temperature set.

Certain details shown in table 5 and figure 4 are of interest. In the first five ten-day periods the high-temperature set gradually increased its fission rate, rising in the fifth ten-day period to a maximum average of 31.76 fissions in ten days for each line, or more than three fissions in 24 hours. During the same five ten-day periods the low-temperature set gradually decreased in fission rate, from an average of 7.8 fissions in ten days, in the first period, to 1.3 fissions in ten days in the fourth and fifth periods. Thus on the fifth ten-day period the high-temperature set averaged 24 fissions to each single fission in the low-temperature set. In the sixth period the fission rate of the high-temperature set dropped precipitately to 18.8 fissions in 10 days. Now it remained with considerable fluctuations till the thirteenth ten-day period, when it again fell off strongly to 14.15 fissions per line. From thence onward it continued to fall rapidly, to 9.7, to 5.6, to 4.7, and finally on the eighteenth period to 1.10 fissions in ten days. During these last periods the high-temperature set was evidently weakened, and many deaths occurred. Five lines died out on the 8th day of the fifteenth ten-day period, and the number of deaths gradually increased until the fifth day of the eighteenth ten-day period, when the last two lines of the high-temperature set died out. Thus in the high-temperature set there were roughly three phases distinguishable. First there was a fifty-day period of increase of fission rate, then seventy days of very gradual decrease; then a final period of sixty days of rapid decrease in fission rate, culminating in the

death of all the lines of the high-temperature set in the 175th day of the culture.

In the set kept at low temperature the fission rate is consistently low, as compared with the other set before it had degenerated. With relation to the changes from week to week in the average fission rate of the low-temperature set, a disturbing factor exists in the fact that it was not possible to keep the low temperature so constant as could be done for the high temperature. Thus the maximum temperature varied somewhat from week to week, as is shown in table 5 (at the left). Examination of these maximum and minimum records of table 5 will show that on

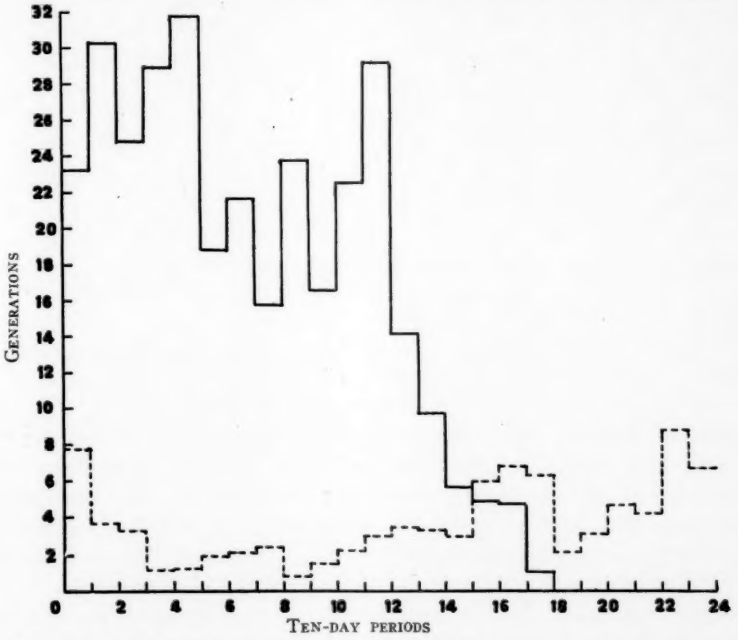


FIGURE 4.—Polygons showing the effects on the fission rate of keeping two sets belonging to the same clone for eight months under opposed extreme temperatures (second series of experiments, basic lines, figure 3, A). The polygons show the average number of generations per line for each 10-day period, the continuous line representing the high-temperature set (kept at 28 to 32 degrees), the broken line the low-temperature set (kept at 6 to 9 degrees). The base line shows the 24 ten-day periods of the experiment, the vertical scale the number of fissions per line. The high-temperature set at first divided much the more rapidly, but fell off till in the 16th period it was below the low-temperature set; at the end of 180 days the high-temperature lines were all dead.

the whole as the maximum for any period is higher, the fission rate for that period is correspondingly higher. There is apparently no other significance to the fact shown in figure 4, that in the low-temperature set the fission rate at first decreased, remained low for about 100 days, then gradually increased, and was highest toward the end. If the temperature could have been kept constant, the fission rate would apparently have remained constant. What *is* significant is that there was no decrease in the fission rate in this set, that no lines died out, and that all remained vigorous and multiplied rapidly until the cultures were discontinued after 240 days.

Thus the experiment indicates that keeping *Stylonychia pustulata* at the high temperature of 28 to 32 degrees centigrade has a cumulative injurious effect,—an effect hardly noticeable till four months have passed, but resulting in two months more in the degeneration and death of all the lines. On the other hand, keeping them at the low temperature (which actually varied from 6 to 15 degrees, and probably averaged about 10 degrees) had no injurious effect; at the end of eight months they were as vigorous as at the beginning.

The question may be raised whether the ultimate death of the high-temperature set was not due to the fact that the heat increased the rate of all vital processes, so that the "life cycle" was passed through more quickly in this set than in the other. The cause of death would then be merely the fact that the vegetative cycle was completed. The total number of fissions in the different lines of the high-temperature set varied from 290 to 378 before extinction occurred. In the same period of time (180 days) the low-temperature lines had each gone through only 53 to 72 fissions; and when the cultures were discontinued with the low-temperature set still vigorous, the total fissions that they had gone through were but 76 (in the lowest line) to 115 (highest line). It might be maintained by anyone that holds to the theory of a definite life cycle limited to a given number of vegetative reproductions, that if the low-temperature set should be kept long enough to attain as many generations as the high-temperature set (which would require about two years), this set too would die out. BAITSELL (1912), however, kept *Stylonychia pustulata* under cultivation for 572 generations, so that it would not seem probable that extinction after 378 generations is due to the completion of the vegetative cycle, even assuming that there is such a cycle. My own experiments give no direct evidence on the point.

(9) *Test of modifications produced after 20 days in diverse temperatures (B, figure 3)*

Progeny from each line of the two sets of the basic experiment were placed, after 20 days in the diverse temperatures, in an intermediate temperature of 16 to 20 degrees, and allowed to multiply there for 20 days. The number of fissions for the entire 20 days, and for the first and second 10-day periods is given for each line of each set in table 6. The like-numbered lines of each set are compared, as in previous tables, and the difference between them given.

As table 6 shows, the set that has been multiplying in the higher temperature continues to multiply the more rapidly after the two sets have been brought to the same temperature. In 23 out of the 30 lines of each set compared, the ex-high line has produced more generations than the ex-low line. The average number of fissions per line for the twenty days is for the ex-high set 29.7, for the ex-low set, 27.6. The average difference is thus but 2.1 fissions in 20 days, but this difference is consistent, showing (as we have seen) in 23 lines out of 30, and being equally evident in the first and second ten days of the culture period.

In this test therefore, as in the two earlier tests of our first series of experiments (pages 541 and 543), the higher fission rate induced by the higher temperature clearly persists in some measure after restoration of the two sets to the same temperature.

(10) *Test of modifications produced after 40 days in diverse temperatures (C, figure 3)*

After one set had been 40 days in the temperature of 28 to 30 degrees, the other in that of 6 to 9 degrees, progeny were again taken from each line of each set and allowed to multiply for 20 days at an intermediate temperature of 14 to 18 degrees. The comparative results are shown in table 7, for the entire period of 20 days, and for each of the two component 10-day periods.

In this test we find a reversal of the conditions found in the previous tests. The set that has been kept at low temperature now divides more rapidly than the set that has been kept at high temperature. In every one of the thirty lines compared from each set, the ex-low set has produced more generations than the ex-high set; and this is true for each of the two ten-day periods taken separately. All but three of the 30 lines of the ex-low set exceed the number of fissions (16) found in the

TABLE 6

SERIES II.—*Test after 20 days (B, figure 3). Number of fissions (generations) for the two sets of thirty lines each kept for 20 days in 16°-20° C., after having been kept for 20 days, one set in 30°-32° C., the other in 6°-10° C., with the excess in favor of the high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																														Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
18°-20° C. ex-high..	15	20	17	18	18	21	20	22	16	15	12	14	15	14	15	15	16	17	14	15	17	16	14	19	16	14	14	16	15	15	485	16.16	
18°-20° C. ex-low...	19	16	14	15	14	16	19	15	14	12	14	13	18	12	14	14	12	15	15	18	16	13	13	18	15	16	16	12	17	16	451	15.03	
Excess	-4	4	3	3	4	5	1	7	2	3	-2	1	-3	2	1	1	4	2	-1	-3	1	3	1	1	1	-2	-2	4	-2	-1	34	1.13	3.63
Second 10-day period																																	
16°-18° C. ex-high..	12	16	14	16	14	13	14	12	14	13	12	14	12	14	16	16	15	16	12	15	11	13	11	15	15	14	13	10	11	13	406	13.53	
16°-18° C. ex-low...	14	12	13	12	14	17	19	17	10	12	14	10	9	13	11	12	11	11	11	15	10	8	10	17	12	12	11	12	13	15	377	12.56	
Excess	-2	4	1	4	0	-4	-5	-5	4	1	-2	4	3	1	5	4	4	5	1	0	1	5	1	-2	3	2	2	-2	-2	-2	29	.97	3.71
Entire 20 days																																	
16°-20° ex-high....	27	36	31	34	32	34	34	34	30	28	24	28	27	28	31	31	31	33	26	30	28	29	25	34	31	28	29	26	26	28	891	29.70	
16°-20° ex-low	33	28	27	27	28	33	38	32	24	24	28	23	27	25	25	26	23	26	26	33	26	21	23	35	27	28	27	24	30	31	828	27.60	
Excess of ex-high....	-6	8	4	7	4	1	-4	2	6	4	-4	5	0	3	6	5	8	7	0	-3	2	8	2	-1	4	0	2	2	-4	-3	63	2.10	3.67

TABLE 7

SERIES II.—*Test after 40 days (C, figure 3). Number of fissions (generations) for the two sets of thirty lines each kept for twenty days in 14° to 18° C. subsequent to forty days in extreme temperatures (28°-32° and 6°-10° C.) with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																													Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
14°-18° C. ex-high..	6	7	6	6	5	6	5	7	6	6	6	7	6	6	6	5	6	6	5	6	5	6	6	5	6	6	6	6	5	6	176	5.87	
14°-18° C. ex-low...	7	8	8	8	7	8	8	7	10	8	9	9	8	9	10	10	7	7	9	8	8	7	9	7	10	8	8	7	7	8	244	8.13	
Excess	-1	-1	-2	-2	-2	-2	-3	0	-4	-2	-3	-2	-2	-3	-4	-5	-1	-1	-4	-2	-3	-1	-3	-2	-4	-2	-2	-1	-2	-2	-68	-2.26	-16.14
Second 10-day period																																	
14°-16° C. ex-high..	10	9	7	10	8	8	8	9	8	9	9	7	9	9	7	8	9	9	9	9	8	9	9	9	9	9	8	8	7	10	257	8.57	
14°-16° C. ex-low...	12	10	11	13	13	14	11	11	12	10	11	11	8	15	12	14	11	9	10	13	9	10	12	13	10	10	9	9	12	14	339	11.30	
Excess	-2	-1	-4	-3	-5	-6	-3	-2	-4	-1	-2	-4	1	-6	-5	-6	-2	0	-1	-4	-1	-1	-3	-4	-1	-1	-1	-1	-5	-4	-82	-2.73	-13.74
Total 20 days																																	
14°-18° ex-high....	16	16	13	16	13	14	13	16	14	15	15	14	15	15	13	13	15	15	14	15	13	15	15	14	15	15	14	14	12	16	433	13.43	
14°-18° ex-low.....	19	18	19	21	20	22	19	18	22	18	20	20	16	24	22	24	18	16	19	21	17	17	21	20	20	18	17	16	19	22	583	19.43	
Excess	-3	-2	-6	-5	-7	-8	-6	-2	-8	-3	-5	-6	-1	-9	-9	-11	-3	-1	-5	-6	-4	-2	-6	-6	-5	-3	-3	-2	-7	-6	-150	-6.00	-14.76

highest line of the ex-high set, and no line of the ex-high set exceeds any line of the ex-low set. The average difference per line is six fissions in twenty days in favor of the ex-low set.

The twenty days of this test are coincident with the fifth and sixth ten-day period of the stock cultures still remaining in the diverse temperatures. It was within this 20-day period that the great falling off took place in the fission rate of the set kept at high temperature (see figure 4). In the fifth ten-day period the average number of fissions per line had been 31.76; in the next period it fell to 18.80. That is, apparently the injury due to the long-continued stay in the high temperature began to be manifest at about this time. It would appear probable that this is connected with the fact shown in our present test (table 7), that when the two sets are subjected to the same intermediate temperature, the ex-high set no longer continues to divide more rapidly than the ex-low set, as had been the case in the previous test. The high-temperature set, on being placed in the lower temperature of 14 to 18 degrees, drop from an average of 50.56 fissions per line in 20 days (table 5, periods 5 and 6) to an average of 13.43 fissions per line in the same period, that is, to about one-fourth their former rate. The low-temperature set, raised to the same intermediate temperature (14 to 18 degrees) increase their average fission rate per line from 3.26 in twenty days to 19.43 in twenty days, that is, to about six times their former rate.

(11) *Test of modifications produced after 60 days in diverse temperatures (D and E, figure 3)*

The reversal of the relative fission rates of the two sets when kept at the same temperature, described in the last section, seemed of such interest as to demand further and thorough study, with variation of the conditions. So at the end of that test, when the basic cultures had been for sixty days in the diverse temperatures, two sets of test progeny were taken from each of the basic lines, and tests were made (a) with both sets of lines at intermediate temperature, and (b) both sets at high temperature. The fission rate was determined in each of these test sets for a period of 60 days. This 60-day period corresponds, as figure 3 shows, with periods 6 to 12, inclusive, of the basic cultures.

Thus at this time (periods 6 to 12, inclusive) there were in progress six sets of cultures, each of 30 lines each; three belonging to the originally high set; three to the originally low set. They formed three pairs of cultures, as follows: (1) A high set still kept at the original temperature of 28 to 30 degrees, with a low set still kept at 6 to 9 degrees; (2)

an ex-high set kept at the intermediate temperature of 14 to 18 degrees, with an ex-low set kept at the same intermediate temperature; (3) a high set kept at 28 degrees, with an ex-low set kept also at this high temperature (28 degrees).

(a) Both sets at the same intermediate temperature (D, figure 3)

During these periods 6 to 12, as figure 4 shows, the high sets of the basic culture still kept up their rapid fission, with little or no indication of injury, while the low-temperature set divided slowly. When the two sets were kept in the intermediate temperature of 14 to 18 degrees, the ex-high set decreased and the ex-low set increased in fission rate to such an extent as to reverse their relative positions, so that the ex-low set now divided more rapidly than the ex-high set. This is the same phenomenon observed in our previous test (section 10). The actual number of fissions for each of the thirty lines of each set is given by 10-day periods in table 8, with comparison of the like-numbered lines. In all of the 180 comparisons thus made, except 2, the ex-low lines divided more rapidly than the corresponding ex-high lines. For the entire period of 60 days, every one of the ex-low lines produced more generations than *any* of the ex-high lines. In every one of the six ten-day periods the total number of generations produced by the thirty ex-low lines was much above the total number produced by the thirty ex-high lines, and the average number of fissions per line per ten-day period was in every case correspondingly greater for the ex-low lines. All these relations are shown in detail in table 8.

Thus this test shows again that cultivation in diverse temperatures has produced in the two halves of the clone a diversity that is inherited in vegetative reproduction. But when the two sets are restored to the same intermediate temperature, the ex-high set, which was dividing the more rapidly, now divides the less rapidly. The inherited effect of the diversity of temperature is the reverse of the direct effect.

(b) Both sets in the same high temperature (E, figure 3)

In this experiment both sets were kept at a temperature about the same as that of the high set in the basic cultures. A different incubator was used, so that the temperature was not absolutely identical with that of the basic high set; apparently it was, for a part of the time at least, a little higher, for the ex-high set divided somewhat more rapidly than did the high set of the basic cultures.

When the two sets were kept at the same high temperature, the ex-low lines increased greatly their fission rate, but during the first ten days it did not reach that of the ex-high set. For these first ten days in 29 out of 30 of the pairs the ex-high line divided more rapidly. The detailed figures are given in table 9. But after these first ten days the ex-low lines increased still further in their fission rate, while the ex-high lines fell off, so that now their positions became reversed (just as happened when the two were in intermediate temperatures). That is, in the last five 10-day periods of this test with both sets at high temperature, the ex-low lines multiplied more rapidly than the ex-high lines. For the entire period of 60 days, every ex-low line produced more generations than any ex-high line. The total number produced in the 30 ex-high lines varied from 78 to 133; the totals in the ex-low lines varied from 148 to 189 generations. Thus the test with both sets at high temperatures gives essentially the same results as that with both sets at intermediate temperatures, save that equilibrium is not reached in the high temperature, until after the first 10-day period.

(12) *Tests of modifications produced after 120 days in diverse tempertures (F, G and H, figure 3)*

At the end of the tests described in section 11, and after the basic lines had been for four months in the diverse temperatures (the high set at 28 to 30 degrees, the low set at 6 to 9 degrees), tests were again made of the permanence of the modifications produced. Three sets of tests were made at this time; (a) with both sets at intermediate temperature (12 to 21 degrees); (b) with both sets at high temperature (28 degrees); (c) with both sets at low temperature (5 to 15 degrees). The tests lasted for 20 days. In this case there were used for each test the progeny of only 20 lines (numbers 11 to 30) of each of the two basic cultures. These three tests coincide in time with periods 13 and 14 of the basic cultures. As figure 4 shows, it was during these two periods that there occurred the extreme decrease in the fission rate of the set kept in the basic cultures at high temperature. The high temperature had apparently produced a marked injurious effect.

In the test at intermediate temperature (F, figure 3, and table 10), and in that at high temperature (G, figure 3, and table 11), the results were the same as in the tests at 40 and at 60 days. The ex-low set divided more rapidly than the ex-high set. In the high-temperature test both sets divided more rapidly than in the intermediate-temperature test, and the result is that in the high temperature the excess in favor of the

TABLE 8

SERIES II.—*Test at intermediate temperature after 60 days (D, figure 3). Number of fissions (generations) for the two sets of thirty lines each kept for sixty days in intermediate (room) temperature subsequent to sixty days in extreme temperatures, with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																															Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																		
Ex-high	6	5	6	3	6	6	6	5	6	8	7	5	5	8	6	5	7	7	7	8	4	5	3	3	4	4	6	4	6	5	166	5.53	
Ex-low	8	9	8	7	6	9	9	11	7	9	8	8	8	8	8	10	8	9	7	8	8	9	8	8	9	9	8	9	9	8	250	8.33	
Excess	−2	−4	−2	−4	0	−3	−3	−6	−1	−1	−1	−3	−3	0	−2	−5	−1	−2	0	0	−4	−4	−5	−5	−5	−5	−2	−5	−3	−3	−84	−2.80	−20.20
Second 10-day period																																		
Ex-high	4	5	3	3	6	7	6	4	6	7	7	8	5	5	5	4	6	3	6	4	2	4	6	4	6	4	3	5	2	4	144	4.80	
Ex-low	9	7	7	6	9	8	9	8	9	8	9	8	10	8	9	8	11	8	10	10	9	7	8	7	9	8	9	8	8	8	252	8.40	
Excess	−5	−2	−4	−3	−3	−1	−3	−4	−3	−1	−2	0	−5	−3	−4	−4	−5	−5	−4	−6	−7	−3	−2	−3	−3	−4	−6	−3	−6	−4	−108	−3.60	−27.27
Third 10-day period																																		
Ex-high	6	3	4	5	8	5	7	6	6	7	6	6	6	6	6	5	6	6	5	4	7	6	7	6	8	4	4	4	5	4	168	5.60	
Ex-low	6	6	7	9	7	8	9	8	7	8	7	8	10	8	8	8	9	10	8	8	8	6	8	8	10	9	9	9	7	7	240	8.00	
Excess	0	−3	−3	−4	1	−3	−2	−2	−1	−1	−1	−2	−4	−2	−2	−3	−3	−4	−3	−4	−1	0	−1	−2	−2	−5	−5	−5	−2	−3	−72	−2.40	−17.65
Fourth 10-day period																																		
Ex-high	3	2	5	1	6	4	5	5	2	6	6	5	5	5	5	6	4	6	5	4	3	4	5	5	6	5	5	4	1	1	129	4.30	
Ex-low	6	6	6	5	6	6	6	8	6	6	8	6	6	6	6	7	6	8	6	7	7	6	6	7	6	7	7	7	7	10	197	6.56	
Excess	−3	−4	−1	−4	0	−2	−1	−3	−4	0	−2	−1	−1	−1	−1	−1	−2	−2	−1	−3	−4	−2	−1	−2	0	−2	−2	−3	−6	−9	−68	−2.26	−20.81
Fifth 10-day period																																		
Ex-high	11	9	9	9	12	15	15	13	12	10	13	13	14	14	14	14	15	14	9	13	9	12	10	12	13	11	12	11	12	12	362	12.06	
Ex-low	12	14	15	20	16	16	18	16	16	15	16	16	16	16	17	16	14	19	16	18	14	16	13	16	16	16	14	15	15	16	473	15.76	
Excess	−4	−5	−6	−11	−4	−1	−3	−3	−4	−5	−3	−3	−2	−2	−3	−2	1	−5	−7	−5	−5	−4	−3	−4	−3	−5	−2	−4	−3	−4	−111	−3.70	−13.29
Sixth 10-day period																																		
Ex-high	6	9	3	7	10	12	10	12	9	9	9	10	11	10	10	9	12	11	8	6	7	11	3	11	11	9	9	8	10	9	271	9.03	
Ex-low	11	12	12	12	11	13	13	13	9	13	12	11	14	14	13	13	12	12	13	12	11	11	14	12	13	11	13	13	15	14	372	12.40	
Excess	−5	−3	−9	−5	−1	−1	−3	−1	0	−4	−3	−1	−3	−4	−3	−4	0	−1	−5	−6	−4	0	−11	−1	−2	−2	−4	−5	−5	−5	−101	−3.37	−15.72
Total, 60 days																																		
Ex-high	36	33	30	28	48	49	49	45	41	47	48	47	46	48	46	43	50	47	40	39	32	42	34	41	48	37	39	36	36	35	1240	41.33	
Ex-low	52	54	55	59	55	60	64	64	54	59	60	57	64	60	61	62	60	66	60	63	57	55	57	58	63	60	60	61	61	63	1784	59.47	
Excess of ex-high	−16	−21	−25	−31	−7	−11	−15	−19	−13	−12	−12	−10	−20	−12	−15	−19	−10	−19	−20	−24	−25	−13	−23	−17	−15	−23	−21	−25	−25	−28	−544	−18.14	−19.14

TABLE 9

SERIES II.—*Test in high temperature (E, figure 3). Number of fissions (generations) for the two sets of lines kept for sixty days in 28° C. subsequent to sixty days with the low lines in 8° C. and the high lines in 28° to 30° C., with the excess in favor of the ex-high sign. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																													Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
First 10-day period																																	
Ex-high	27	26	27	24	26	25	27	27	27	30	28	28	28	24	28	24	27	27	23	25	25	26	27	27	27	28	27	23	25	25	788	26.26	
Ex-low	20	23	21	20	21	21	22	24	24	18	21	21	22	21	22	25	22	23	23	22	22	22	21	21	24	23	24	20	23	23	659	21.96	
Excess	7	3	6	4	5	4	5	3	3	12	7	7	6	3	6	−1	5	4	0	3	3	4	6	6	3	5	3	3	2	2	129	4.30	8.91
Second 10-day period																																	
Ex-high	13	18	19	17	22	20	20	31	20	16	19	21	12	12	17	14	10	11	9	12	13	15	18	17	10	18	14	17	12	14	481	16.03	
Ex-low	28	28	29	27	25	26	25	26	28	27	22	25	26	25	26	24	23	24	27	25	27	24	25	27	26	26	24	27	27	27	776	25.86	
Excess	−15	−10	−10	−10	−3	−6	−5	5	−8	−11	−3	−4	−14	−13	−9	−10	−13	−13	−18	−13	−14	−9	−7	−10	−16	−8	−10	−10	−15	−13	−295	−9.83	−23.46
Third 10-day period																																	
Ex-high	12	17	18	21	18	13	14	25	13	14	17	17	8	12	10	26	13	10	10	4	10	12	6	13	9	9	10	12	4	8	390	13.00	
Ex-low	28	23	30	26	28	28	30	33	25	25	27	25	31	31	23	30	29	31	26	26	30	27	28	27	27	28	29	29	26	28	834	27.80	
Excess	−16	−6	−12	−5	−10	−15	−16	−8	−7	−11	−10	−8	−23	−19	−13	−4	−16	−21	−16	−22	−20	−15	−22	−14	−18	−19	−19	−17	−22	−20	−444	−14.80	−36.27
Fourth 10-day period																																	
Ex-high	8	9	9	10	11	12	13	19	17	12	22	20	16	16	8	16	18	17	15	14	9	6	9	10	8	9	6	9	16	16	380	12.66	
Ex-low	25	24	26	30	22	24	26	27	25	27	26	26	22	25	26	28	25	27	24	23	23	24	24	24	26	24	23	24	23	20	743	24.76	
Excess	−17	−15	−17	−20	−11	−12	−13	−8	−8	−15	−4	−6	−6	−9	−18	−12	−7	−10	−9	−9	−14	−18	−15	−14	−18	−15	−17	−15	−7	−4	−363	−12.10	−32.33
Fifth 10-day period																																	
Ex-high	19	19	22	21	17	18	21	12	20	20	21	17	20	20	19	14	21	16	17	19	11	12	13	11	9	16	5	10	12	15	487	16.23	
Ex-low	41	39	38	49	41	40	41	39	35	41	36	41	38	40	41	38	37	34	41	32	42	42	42	48	46	40	44	40	43	45	1214	40.46	
Excess	−22	−20	−16	−28	−24	−22	−20	−27	−15	−21	−15	−24	−18	−20	−22	−24	−16	−18	−24	−13	−31	−30	−29	−37	−37	−24	−39	−30	−31	−30	−727	−24.23	−42.74
Sixth 10-day period																																	
Ex-high	14	17	14	12	14	14	14	7	12	10	17	19	12	12	6	6	12	14	14	10	15	7	7	13	15	15	17	15	13	12	393	13.10	
Ex-low	27	27	30	29	29	26	33	31	30	30	33	33	36	35	35	30	26	27	34	29	36	37	30	35	31	31	30	32	32	32	936	31.20	
Excess	−13	−10	−16	−17	−15	−12	−19	−24	−18	−20	−16	−14	−24	−23	−29	−24	−14	−13	−20	−19	−21	−30	−23	−22	−16	−16	−13	−17	−19	−20	−543	−18.10	−40.85
Total, 60 days																																	
Ex-high	93	106	109	105	108	102	109	121	114	102	124	122	96	96	88	100	101	95	88	84	83	78	80	91	78	95	79	86	82	90	2919	97.30	
Ex-low	169	164	174	181	166	165	177	180	167	168	165	171	175	177	173	175	162	166	175	157	180	176	170	182	180	172	174	172	174	175	5162	172.07	
Excess	−76	−58	−65	−76	−58	−63	−68	−59	−53	−66	−41	−49	−79	−81	−85	−75	−61	−71	−87	−73	−97	−98	−90	−91	−102	−77	−96	−86	−92	−85	−2243	−74.77	−27.76

TABLE 10

SERIES II.—*Test in intermediate temperature (F, figure 3). Number of fissions (generations) for the last twenty lines of the two sets of lines kept for twenty days in intermediate temperature subsequent to one hundred and twenty days in extreme temperatures; with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																					Total	Average per line	Percent that the difference is of the total in all
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
First 10-day period																								
Ex-high	4	4	2	4	5	6	4	6	3	7	2	7	5	3	5	5	4	7	3	5		91	4.55	
Ex-low	7	8	14	14	13	14	14	13	12	14	9	12	10	10	10	10	13	14	11	10		232	11.60	
Excess	-3	-4	-12	-10	-8	-8	-10	-7	-9	-7	-7	-5	-5	-7	-5	-5	-9	-7	-8	-5		-141	-7.05	-43.65
Second 10-day period																								
Ex-high	6	1	3	2	4	4	8	7	4	5	6	7	4	3	8	5	4	4	5	5		95	4.75	
Ex-low	7	8	13	14	14	13	12	13	12	13	11	13	10	10	9	12	13	14	14	15		240	12.00	
Excess	-1	-7	-10	-12	-10	-9	-4	-6	-8	-8	-5	-6	-6	-7	-1	-7	-9	-10	-9	-10		-145	-7.25	-43.24
Total, 20 days																								
Ex-high	10	5	5	6	9	10	12	13	7	12	8	14	9	9	13	10	8	11	8	10		186	9.30	
Ex-low	14	16	27	28	27	27	26	26	24	27	20	25	20	20	19	22	26	28	25	25		472	23.60	
Excess	-4	-11	-22	-22	-18	-17	-14	-13	-17	-15	-12	-11	-11	-11	-6	-12	-18	-17	-17	-15		-286	-14.30	-43.47

TABLE II

SERIES II.—*Test at high temperature (G, figure 3). Number of fissions (generations) for each of the two sets kept for twenty days in high temperature (28° C.) subsequent to 120 days in extreme temperatures; with the excess in favor of the high set. (Differences given the minus sign show an excess of the low set.)*

	Lines number																					Total	Average per line	Percent that the difference is of the total in all
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
First 10-day period																								
Ex-high	11	10	8	7	21	18	22	23	16	13	10	10	15	14	9	15	15	18	12	16		283	14.15	
Ex-low	25	26	34	37	29	34	30	36	37	35	26	30	29	32	26	33	31	30	28	29		617	30.85	
Excess of ex-high	-14	-16	-26	-30	-8	-16	-8	-13	-21	-22	-16	-20	-14	-18	-17	-18	-16	-12	-16	-13		-334	-16.70	-37.11
Second 10-day period																								
Ex-high	4	7	6	10	9	6	9	11	10	10	8	8	7	8	8	20	13	22	11	7		194	9.70	
Ex-low	33	35	32	42	39	38	32	35	33	35	30	34	30	32	33	32	31	35	32	31		674	33.70	
Excess of ex-high	-29	-28	-26	-32	-30	-32	-23	-24	-23	-25	-22	-26	-23	-24	-25	-12	-18	-13	-21	-24		-480	-24.00	-55.32
Total 20 days																								
Ex-high	15	17	14	17	30	24	31	34	26	23	18	18	22	22	17	35	28	40	23	23		477	23.85	
Ex-low	58	61	66	79	68	72	62	71	70	70	56	64	59	64	59	65	62	65	60	60		1291	64.55	
Excess of ex-high	-43	-44	-52	-62	-38	-48	-31	-37	-44	-47	-38	-46	-37	-42	-42	-30	-34	-25	-37	-37		-814	-40.70	-46.04

ex-low lines is absolutely greater. But relative to the actual rate of fission in the two cases the results are much the same, as shown by the percentage that the excess is of the total fissions (tables 10 and 11). In both of these tests every ex-low line produced during the 20 days more generations than any ex-high line.

In the test with both sets at low temperature (5 to 15 degrees), (H, figure 3) the results were peculiar. The ex-high set on being placed in the low temperature divided several times on the first day, the 20 lines giving 69 fissions during the first day of low temperature. After this the ex-high set almost completely ceased to divide. During the other nine days of the first ten-day period there were but 12 fissions among the twenty lines of the ex-high set, and during the second ten-day period a total of but 7 fissions occurred in this set. During the second ten-day period many of the ex-high lines died, and at the end of the 20 days in low temperature only seven of the original twenty remained. Meanwhile, the ex-low set were dividing normally; during the first 10 days they showed 67 fissions; during the second 10 days, 65 fissions.

Thus it appears clear that long-continued cultivation at high temperature had so affected the animals that they could not survive continued exposure to low temperatures. Aside from the burst of reproduction in the ex-high set on the first day of exposure to low temperature, in this test as in the others the ex-low set multiplies much more vigorously than the ex-high set.

Throughout these three tests, therefore, it is clear that when the sets living under high and low temperatures respectively are placed at the same temperature (whether this be high, low or intermediate), the ex-low set are more vigorous and multiply more rapidly than the ex-high set. The high temperature has injured the vitality and reproductive power of the set exposed to it for a long period, while the low temperature has had no such effect.

(13) *Test of the modifications after 170 days in diverse temperatures (K, figure 3)*

After 5 months and 20 days in the diverse temperatures, the set exposed to the high temperature had become much weakened, and was now multiplying less rapidly than the set at low temperature (see table 5, and figure 4). Progeny of the two sets were now placed in an intermediate temperature of 18 to 20 degrees. This failed to revive the high temperature set. During 10 days the 20 ex-high lines gave a total of but five fissions, and all were dead before the 10 days were up. The 20

lines of the ex-low set meanwhile divided vigorously, producing 325 fissions in the 10 days.

(14) *General results of the second series of experiments*

This very extensive second series of experiments shows that when one set of lines of Stylonychia are kept for a long time at a temperature as high as 28 to 30 degrees, the other at 6 to 9 degrees, the set at the high temperature at first divides much more rapidly than the other. But after some months at the high temperature the vigor of the high-temperature stock is gradually undermined and destroyed; their rate of multiplication becomes less, finally falling below that of the low-temperature set; many of the lines die out, and at the end of six months all are dead. The low-temperature set on the other hand continue their slow rate of division, and show no indications of decreased vitality at the end of eight months.

After but 20 days in these diverse temperatures, if the two are brought to the same intermediate temperature, the set that had been in the high temperature continue to divide more rapidly than the set that had been in low temperature. This confirms the result of the first series of experiments. But after the two sets have been in the diverse temperatures for longer periods (forty days or more), then when the two are brought to the same temperature, the set that have been kept at the lower temperature (and were dividing more slowly), now divides more rapidly than the other. This is true whether the common temperature at which the two are cultivated is high, intermediate or low. It was true in tests made after 40 days; after 60 days; after 120 days, and after 170 days. The different fission rate thus induced lasted for at least six weeks after the two sets were restored to the common temperature.

Thus exposure to diverse temperature induces in a single stock diversities that are heritable in vegetative reproduction. After a limited period the inherited effect is manifest in the same direction as the immediate effect of the different temperature. After a longer period the inherited effect on the fission rate is the reverse of the immediate effect.

THIRD SERIES OF EXPERIMENTS

(15) *General outline* (*figure 5*)

The results of the first and second series of experiments were further tested by a relatively brief third series, lasting all together 60 days. A diagram of the tests made in this series is shown in figure 5.

On April 10, 1916, forty animals of the sixth filial generation from a single individual propagating asexually, taken from a 'wild' laboratory culture of *Stylonychia pustulata,* were isolated in the usual way. Of the forty lines of propagation obtained from these, twenty were kept at a high temperature of 24 to 27 degrees; the other 20 at a low temperature of 8 to 18 degrees. They were cultivated at these diverse temperatures for sixty days. These form the basic cultures for this series (A, figure 5).

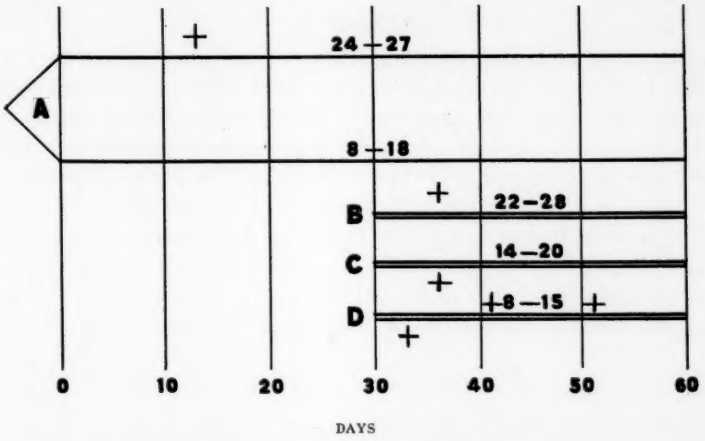


FIGURE 5.—Diagram of the third series of experiments. The horizontal lines represent the duration of the cultures; the vertical lines show successive 10-day periods.

A. The two basic cultures, derived from a single parent, and kept for 60 days at the diverse temperatures shown by the numbers above the lines. B, C and D, tests with progeny of both the basic sets under the same temperature, after 30 days in opposite extreme temperatures. B, test at high temperature; C, at intermediate temperature; D, at low temperature.

A + sign above the test lines shows that the high-temperature set (or ex-high set) divides more rapidly; below the test lines it shows that the low-temperature set (or ex-low) divides more rapidly.

After thirty days in these diverse temperatures, three tests were made. In one, lines from both the basic sets were kept at a high temperature (B, figure 5); in the second both sets were kept at an intermediate temperature (C, figure 5); in the third both sets were kept at a low temperature (D, figure 5). In all these six sets (twenty lines in each set) the fission rate was determined for thirty days. The tests were coincident in time with the last thirty days of the basic cultures.

(16) *The basic cultures, kept for 60 days at diverse temperatures (A, figure 5)*

The number of fissions for each of the twenty lines is shown for each of the six 10-day periods of the experiment in table 14. On the whole the lines kept at the high temperature increased their fission rate in the later periods, while those in the lower temperature decreased their rate. There was some fluctuation in the temperatures from period to period, as table 12 shows, and it is not improbable that the variations in fission rate were directly correlated with these. The important points in the records are that there was a very great difference between the two sets in respect to their fission rates, and that there is no indication of any injurious effect of either the high or of the low temperature.

(17) *Tests after 30 days at diverse temperatures*

(a) Test at high temperature (B, figure 5)

In the two sets kept at 22 to 28 degrees, those that had before been kept at higher temperature continued to divide more rapidly than the set that had been kept at low temperature. The fissions for each line of each set for the three ten-day periods of the test are shown in table 13. Out of the sixty comparisons made, in all but four the ex-high set divided more rapidly than the ex-low set. In the entire period of 60 days, the lowest line of the ex-high set produced more generations than the highest line of the ex-low set. Thus the two sets derived from the same parent, and kept for these thirty days under the same temperature, are distinctly diverse in their fission rate, as a result of the earlier diversity of temperature under which they lived.

It is notable that in the last ten-day period of the thirty days at the same temperature, the difference between the two sets did not become less. It was in fact somewhat greater in the last 10 days.

TABLE 12

SERIES III.—*Basic cultures (A, figure 5). Number of fissions (generations) for the two sets of twenty lines each; one set kept for 60 days in high temperature, the other set in low temperature, with the excess in favor of the high set. (Differences given the minus sign below show an excess of the low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																							
24° to 26° C. High	16	14	13	15	16	16	15	14	14	15	17	16	16	16	15	15	16	15	14	16	304	15.20	
8° to 11° C. Low	2	1	2	3	3	5	3	1	3	2	3	1	2	1	3	1	3	1	3	1	44	2.20	
Excess	14	13	11	12	13	11	12	13	11	13	14	15	14	15	12	14	13	14	11	15	260	13.00	75.86
Second 10-day period																							
24.5° to 26° C. High	22	20	19	20	24	22	21	20	25	23	26	21	23	21	21	22	22	22	24	23	441	22.05	
8.5° to 12° C. Low	5	5	2	4	5	5	4	3	4	5	3	3	4	4	4	1	4	4	4	4	77	3.85	
Excess	17	15	17	16	19	17	17	17	21	18	23	18	19	17	17	21	18	18	20	19	364	18.20	70.27
Third 10-day period																							
24.5° to 27° C. High	19	18	20	17	22	19	22	19	25	21	22	21	21	19	18	20	18	21	20	19	401	20.05	
9.5° to 17° C. Low	8	7	7	8	7	7	7	6	6	8	4	7	6	7	5	3	7	7	6	6	129	6.45	
Excess	11	11	13	9	15	12	15	13	19	13	18	14	15	12	13	17	11	14	14	13	272	13.60	51.32
Fourth 10-day period																							
24° to 26.5° C. High	18	15	17	14	16	15	19	18	17	16	16	16	18	14	15	16	15	16	14	15	320	16.00	
10° to 16 ° C. Low	8	10	5	7	9	7	7	6	8	9	8	8	8	7	7	8	8	8	10	10	158	7.90	
Excess	10	5	12	7	7	8	12	12	9	7	8	8	10	7	8	8	7	8	4	5	162	8.10	33.67
Fifth 10-day period																							
25° to 27° C. High	17	14	14	17	20	19	22	17	20	30	19	29	18	31	20	23	22	23	17	14	406	20.30	
8° to 18° C. Low	2	4	4	4	2	4	2	2	3	3	1	2	4	3	3	3	3	3	2	3	57	2.85	
Excess	15	10	10	13	18	15	20	15	17	27	18	27	14	28	17	20	19	20	15	11	349	17.45	75.37
Sixth 10-day period																							
21.5° to 27° C. High	26	25	24	22	25	25	21	17	22	27	21	27	25	27	26	24	29	28	26	26	593	29.65	
8° to 13° C. Low	3	2	3	2	1	2	5	3	5	3	5	3	3	3	0	1	5	3	5	2	60	3.00	
Excess	23	23	21	20	24	23	16	14	17	24	16	24	22	24	26	23	24	25	21	24	533	26.65	81.62
Total, 60 days																							
High	118	106	107	105	123	116	120	105	123	132	121	130	121	128	115	120	122	125	115	113	2465	123.25	
Low	28	29	23	28	27	30	28	21	29	30	24	24	27	25	22	17	30	26	30	26	525	26.25	
Excess	90	77	84	77	96	86	92	84	94	102	97	106	94	103	93	103	92	99	85	87	1940	97.00	64.88

(b) Test at intermediate temperature (C, figure 5)

When the two sets that had lived for thirty days under diverse temperatures were placed in the same intermediate temperature of 14 to 20 degrees, the set that had been kept at the lower temperature divided somewhat more rapidly than the set that had been kept at high temperature. The exact figures are given in table 14. The difference is not very great, but is constant. In only three cases out of a possible forty in the first and second ten-day periods did the ex-high line show an excess over the corresponding ex-low line, and in only five cases out of a possible twenty in the third period. In the entire 60 comparisons made in table 14, the ex-low line showed the higher fission rate in 43. For the entire period of 60 days the ex-high lines varied from 26 to 40 fissions, with the average at 30.45, while the ex-low lines varied from 31 to 42, with the average at 36.30.

Thus when the two are tested at intermediate temperatures they give a result the reverse of that reached when tested at high temperatures. In the high temperature the set that have before been at high temperatures multiply faster; in the intermediate temperature those that have before been at low temperature multiply faster.

(c) Test at low temperature (D, figure 5)

The two sets that had been living at diverse temperatures for thirty days were both placed under a common low temperature of 10 to 15 degrees, (D, figure 5). During the first 10-day period of the test the set that had been living under low temperature multiplied distinctly more rapidly than the ex-high set (table 15). Of the 20 pairs compared, in all but four the fission rate was greater in the member from the ex-low set. In the second and third ten-day periods however, the ex-high lines multiplied more rapidly than the ex-low lines. The difference is not so great as the opposite difference in the first ten-day period, but appears uniform enough to be significant (see table 15). This result is difficult to interpret.

(18) *General results of the third series of experiments*

The results of this relatively brief third series of experiments are not entirely clear. But in the earlier series of experiments we had found that when the two sets had been cultivated for so short a period as twenty days, on a test under common conditions the ex-high set retain their higher rate of fission; while after forty days they fall, when tested, behind the other set. In the present series the exposure had been for

TABLE 13

SERIES III.—*Test at high temperature (B, figure 5). Number of fissions (generations) for the two sets of twenty lines each, both kept for thirty days in high temperature, 22°-27° C., subsequent to 30 days in extreme temperatures, 21°-27° C. and 8°-17° C.; with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																							
Ex-high	18	15	17	14	16	15	19	18	17	16	16	17	19	15	16	16	15	16	14	15	324	16.20	
Ex-low	12	12	12	11	11	10	12	13	11	11	12	11	12	12	10	9	12	9	14	13	229	11.45	
Excess	6	3	5	3	5	5	7	5	6	5	4	6	7	3	6	7	3	7	0	2	95	4.75	17.17
Second 10-day period																							
Ex-high	17	14	14	17	20	19	22	17	20	30	19	29	18	31	20	23	22	23	17	14	407	20.35	
Ex-low	14	12	8	9	12	15	15	27	18	32	12	14	18	15	13	12	15	14	17	17	309	15.45	
Excess	3	2	6	8	8	4	7	−10	2	−2	7	15	0	16	7	11	7	9	0	−3	98	4.90	13.68
Third 10-day period																							
Ex-high	26	25	24	22	25	25	21	17	22	27	21	27	25	27	26	24	29	28	26	26	493	24.65	
Ex-low	19	21	18	18	20	23	21	26	22	27	15	17	18	19	18	17	18	18	17	16	388	19.40	
Excess	7	4	6	4	5	2	0	−9	0	0	6	10	7	8	8	7	11	10	9	10	105	525	11.91
Total, 30 days																							
Ex-high	61	54	55	53	61	59	62	52	59	73	56	73	62	73	62	63	66	67	57	55	1224	61.20	
Ex-low	45	45	38	38	43	48	48	66	51	70	39	42	48	46	41	38	45	41	48	46	926	46.30	
Excess	16	9	17	15	18	11	14	−14	8	3	17	31	14	27	21	25	21	26	9	9	298	14.90	13.86

TABLE 14

SERIES III.—*Test at intermediate temperature (C, figure 5). Number of fissions (generations) for the two sets of twenty lines each, both kept for thirty days in intermediate temperature, 14° to 20° C., subsequent to thirty days in extreme temperatures (21° to 27° C. and 8° to 17° C.); with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																							
Ex-high	12	10	11	11	11	11	14	9	7	12	10	8	11	9	9	11	10	9	9	9	203	10.15	
Ex-low	12	12	11	12	11	11	13	12	10	10	14	13	13	13	16	13	16	15	12	10	249	12.45	
Excess	0	−2	0	−1	0	0	1	−3	−3	−2	−4	−5	−2	−4	−7	−2	−6	−6	−3	−1	−46	−2.30	−10.17
Second 10-day period																							
Ex-high	10	10	9	9	12	8	16	7	11	14	12	10	10	11	9	11	8	9	10	9	205	10.25	
Ex-low	15	16	9	11	15	14	15	14	12	12	8	13	13	15	12	12	14	11	11	13	255	12.75	
Excess	−5	−6	0	−2	−3	−6	1	−7	−1	2	4	−3	−3	−4	−3	−1	−6	−2	−1	−4	−50	−2.50	−10.86
Third 10-day period																							
Ex-high	9	10	9	11	9	11	13	11	14	10	9	11	8	12	9	8	8	8	8	13	201	10.05	
Ex-low	12	10	14	11	11	12	10	10	9	10	12	10	10	14	11	14	10	12	10	10	222	11.10	
Excess	−3	0	−5	0	−2	−1	3	1	5	0	−3	1	−2	−2	−2	−6	−2	−4	−2	3	−21	−1.05	−4.96
Total, 30 days																							
Ex-high	31	30	29	31	32	30	43	27	32	36	31	29	29	32	27	30	26	26	27	31	609	30.45	
Ex-low	39	38	34	34	37	37	38	36	31	32	34	36	36	42	39	39	40	38	33	33	726	36.30	
Excess	−8	−8	−5	−3	−5	−7	5	−9	1	4	−3	−7	−7	−10	−12	−9	−14	−12	−6	−2	−117	−5.85	−8.69

TABLE 15

SERIES III.—*Test at low temperature (D, figure 5). Number of fissions (generations) for the two sets of twenty lines each, both kept for thirty days in low temperature, 8° to 15° C., subsequent to thirty days in extreme temperatures (21° to 27° C. and 8° to 17° C.) with the excess in favor of the ex-high set. (Differences given the minus sign show an excess of the ex-low set.)*

	Lines number																				Total	Average per line	Percent that the difference is of the total in all
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
First 10-day period																							
Ex-high	6	6	6	5	6	7	8	6	7	7	6	5	5	5	5	6	4	6	5	6	117	5.85	
Ex-low	8	5	10	7	9	7	7	6	8	9	8	8	8	7	7	8	8	8	10	10	158	7.90	
Excess	−2	1	−4	−2	−3	0	1	0	−1	−2	−2	−3	−3	−2	−2	−2	−4	−2	−5	−4	−41	−2.05	−14.90
Second 10-day period																							
Ex-high	4	4	5	4	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	4	67	3.35	
Ex-low	2	4	4	4	2	4	2	2	3	3	1	2	4	3	3	3	3	3	2	3	57	2.85	
Excess	2	0	1	0	1	−1	2	1	0	0	2	1	−1	0	0	0	0	0	1	1	10	.50	+8.06
Third 10-day period																							
Ex-high	5	4	5	5	4	3	2	4	4	4	3	3	4	5	3	4	4	4	3	5	78	3.90	
Ex-low	3	2	2	3	2	2	5	3	5	3	5	3	3	3	0	1	5	3	5	2	60	3.00	
Excess	2	2	3	2	2	1	−3	1	−1	1	−2	0	1	2	3	3	−1	1	−2	3	18	.90	+13.04
Total, 30 days																							
Ex-high	15	14	16	14	13	13	14	13	14	14	12	11	12	13	11	13	11	13	11	15	262	13.10	
Ex-low	13	11	16	14	13	13	14	11	16	15	14	13	15	13	10	12	16	14	17	15	275	13.75	
Excess	2	3	0	0	0	0	0	2	−2	−1	−2	−2	−3	0	1	1	−5	−1	−6	0	−13	−.65	−2.40

the intermediate period of thirty days, when apparently one of these conditions was transforming into the other. It is therefore perhaps not surprising that the results are not uniform.

GENERAL RESULTS AND CONCLUSIONS

All the experiments show that when infusoria of the same stock are cultivated for considerable periods under diverse temperatures, there result differences between the two sets that are hereditary under vegetative fission. When the two sets are restored to the same temperature, they continue to show diverse fission rates, and this for long periods.

The precise nature of the differences depends upon the length of the period during which the two sets have been kept at different temperatures. After a relatively short period (twenty days or somewhat more), when the two are transferred to a common intermediate temperature, the set that has been at the higher temperature continues to divide more rapidly. But after long periods of exposure to high and low temperatures, when the two are placed at a common temperature the ex-low set divides more rapidly than the ex-high set. The difference thus produced is very great, and is uniform. Apparently the vigor of the organisms is impaired by long continuance of high temperatures, while cultivation at low temperatures has no such effect. Thus on restoration to common intermediate temperatures the ex-low set take and hold the lead in reproduction.

If the cultivation in high temperatures (30 to 32 degrees) is prolonged to six months, the organisms all die, while the low-temperature cultures (6 to 9 degrees) continue to flourish. The latter showed no signs of injury when the cultures were discontinued at the end of eight months.

The most interesting result is perhaps that which follows when the exposure to diverse temperatures has not been so prolonged as to injure the high-temperature set. In this case the diversity in fission rate impressed upon the organisms by the direct action of the different conditions is continued after the conditions have become the same for both sets. But after injury has been induced by high temperature, the inherited effect is manifested in a reversal of the direct action of the different conditions.

It would be of great interest to produce diversities of fission rate by other means, particularly by diverse chemical action, and to determine the inherited results of these. Our results suggest that the numerous

lines or races differing slightly in inherited characteristics, which seem to occur in all lower organisms, have been produced in part by past diverse environmental conditions.

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ON A BACK CROSS IN MICE INVOLVING THREE ALLELOMORPHIC PAIRS OF CHARACTERS[1]

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INTRODUCTION

According to current conceptions in genetics, any two Mendelian factors may bear to each other one of the following relationships: allelomorphic, independent, or linked. The relationship presumably depends upon the locus of the factors in a particular pair of chromosomes. Numerous instances of the first two types of relationship have been found in mammals. Many data bearing on linkage in Drosophila, silkworms, sweet peas, snapdragons and stocks, have prompted the search for linkage in mammals. Up to the present time, the following cases of linkage in mammals have been shown or suggested: the red-eye and pink-eye factors in rats (CASTLE and WRIGHT 1915, 1916), the red-eye and color factors in rats, the pink-eye and color factors in rats (CASTLE and

[1] Paper No. 6, from the Laboratory of Genetics, Department of Animal Husbandry, ILLINOIS AGRICULTURAL EXPERIMENT STATION.

WRIGHT 1916), and the pink-eye and color factors in mice (DARBISHIRE 1904; HALDANE, SPRUNT and HALDANE (1916).

There are several reasons why linkage has not been abundantly observed in mammals. Unless linkage is of a rather pronounced type, the numbers available in dihybrid or multihybrid crosses are usually inadequate to disclose the fact. Whenever linkage between two characters is weak, positive association easily escapes our attention; for, as the $n:1:1:n$ gametic ratio of coupling approaches the $1:1:1:1$ gametic ratio of simple Mendelian segregation, it becomes increasingly difficult to distinguish between these two cases. Furthermore, the usual plan of mating the F_1 individuals *inter se* tends to magnify disparity in the size of segregating classes and to obscure linkage, so that it is not seen directly but must be calculated. However, back-crossing the F_1 to the ultimate recessive gives a zygotic ratio which is an immediate index of the gametic ratio, and does not magnify disparity in the size of segregating classes. The former type of mating often gives F_2 classes so extremely large and small that one hesitates to apply any measure of goodness of fit. The advantages of back-crossing are seen in the ease with which coupling is disclosed in sex-linked inheritance in Drosophila because requisite back-crossing gives F_2 males which immediately indicate the gametic ratio of heterozygous F_1 females. In the absence of any apparent linkage, we look for Mendelian inheritance,—segregation of allelomorphs with chance recombination of independent factors in gametogenesis and chance fertilization of gametes, subject to fluctuations of sampling. To be sure, we may fail to obtain either clear evidence of coupling or satisfactory Mendelian ratios, and we may then account for the discrepancy on the assumption of selective elimination of certain zygotic classes, although not always altogether convincing.

The purpose of the present study is: (1) to apply a more sensitive test for linkage in the relation of agouti, dark eye, and black to each other by the use of back crosses; (2) to study the effect of elimination upon F_2 ratios involving these characters; and (3) to study fluctuations of sampling. The first two studies are embodied in the present paper, but the third will be dealt with in a subsequent paper.

Three wild gray male mice were bred to 24 pink-eyed intense brown females producing 324 F_1 individuals, of which 268 (126 males + 142 females) lived to be classified as wild gray. The F_1 generation was bred back to the triple recessive parent type, the pink-eyed intense brown. All of the F_1 females were examined at five-day intervals and each pregnant female was removed to a separate pen; hence each litter was born in a

separate pen and there is no doubt as to parentage. Each pregnancy pen was examined daily until the birth record was taken. The young were classified when about two weeks old, and reclassified a week later. Whenever pink-eyed black agouti or pink-eyed brown agouti types were involved, they were reclassified several times to avoid confusing two varieties which may appear similar at an early age but are clearly differentiated at four weeks. All litters from F_1 females can therefore be classified as undepleted or depleted. The undepleted are those in which the total number recorded at birth were classified; but in the depleted, deaths occurred between birth record and classification. The number of full litters is greatly increased by isolating pregnant females. Of 531 litters only 170 showed any depletion. We have made no attempt to subdivide the depleted litters according to the causes of elimination. When elimination is due to destruction by mothers, it is undoubtedly unselective and should have no effect upon the F_2 ratios. But when elimination is due to poor care, or neglect by the dam, or to disease and the like, elimination may be selective with regard to Mendelian classes. Since poor ratios have been attributed to elimination, we made an effort to distinguish between these two types of litters. The possibility exists that some elimination may have occurred between the actual birth and the time of birth record, for it might be supposed that young died and were entirely eaten during this interval. We are inclined to put little weight on this possibility for each pregnant female was nested in clean white paper excelsior, and when young died or were eaten, very evident remains disclosed this fact. The interim between actual birth and birth record was not more than 24 hours, and was usually much less. The short time between actual birth and the time of birth record, the excellent conditions under which births occurred, and the absence of traces of elimination, lead us to believe that depletion is readily detected and undepleted litters are easily recognized. We have been dealing entirely with post-natal elimination. Prenatal elimination also occurs in mice as is shown by the presence of atrophic foetuses in the uterus of pregnant females. Whether or not selection is involved here, we do not know; but the results of our experiment indicate that there is no prenatal selection with regard to the Mendelian classes studied.

In Mendelian symbols the parents are wild gray, *AADDBB*, and pink-eyed brown, *aaddbb*. The F_1 hybrids, wild gray in appearance, are *AaDdBb*. The symbols have the following significance:

A, the agouti factor, which restricts black or brown pigment in the subapical yellow band in the individual hairs, producing the

ticked or agouti pattern. Its allelomorph, the absence of ticking, is designated by *a*.

D, the factor for the dark or pigmented eye. Its allelomorph is the pink-eyed condition, *d*. A reduction in the amount of black and brown in the hair goes with the pink-eyed condition.

B, the factor for black pigment. Its allelomorph is brown, *b*.

The F_2 generation obtained by back-crossing the F_1 hybrids to the triple recessive should give in equal numbers the following classes:

AaDdBb, black agouti, dark-eyed	*AaddBb*, black agouti, pink-eyed
AaDdbb, brown agouti " "	*Aaddbb*, brown agouti, " "
aaDdBb, black, " "	*aaddBb*, black, " "
aaDdbb, brown, " "	*aaddbb*, brown, " "

The cross, taken as a whole, is a trihybrid cross, and is dealt with as such in table 3. It is convenient, however, to discuss the results in the following order: monohybrid ratios (table 1), dihybrid ratios (table 2), trihybrid ratios (table 3). Tables 1 and 2 are derived entirely from the ultimate class frequencies of table 3, the data being grouped into monohybrid and dihybrid ratios, a perfectly legitimate procedure inasmuch as we are making the ratios a test against Mendelian theory. Each table contains several ratios, for the class frequencies from four distinct sources are tabulated separately. The four sources of data are indicated in each table and are always given in the following order:

F_1 females giving F_2 young in undepleted litters.
F_1 females giving F_2 in depleted litters.
F_1 males giving F_2 young in stock pens.
F_2 females (triple hybrids) giving F_3 young in stock pens.

Ninety F_1 females produced 531 litters in separate pens, of which 361 were undepleted and 170 were depleted. Two F_1 males, and a number of F_2 females were back-crossed in the stock pens, and the young were classified once a week. No account was taken of elimination between birth and classification. The litters may therefore consist of both depleted and undepleted types. The eight types of F_2 female segregates were likewise tested out in stock pens. With the exception of the F_2 trihybrid wild gray females, these results are tabulated in tables 4 and 5. The total number of segregates classified in all of our experiments is 5566.

It may be stated finally that throughout our experiments the pink-eyed brown (ultimate recessive) is one parent in every mating described

in this paper. If independent allelomorphic pairs are involved, segregating classes should theoretically be of equal frequency. This is an advantage in the use of the formula $\sigma = \sqrt{npq}$ in our experiments. This ideal case, when $p = q = \frac{1}{2}$ and n is large, is not open to the objections frequently raised to its use in many Mendelian ratios. Simply stated, we are always making our ratios a test against a binomial distribution which shows perfect symmetry, and we may therefore make use of the probability integral tables to determine odds against observed deviations without hesitation.

It is misleading to pool data, when subgroups show excessively wide deviation but counterbalance each other. We are not aware of this inaccuracy in our data. We have pooled LITTLE and PHILLIPS's (1913) data for comparison although such a course is not wholly justified in this case.

MONOHYBRID RATIOS

Since there are three allelomorphic pairs involved, there are three sets of monohybrid ratios; agouti: non-agouti, dark eye: pink eye, and black: brown. These ratios are given in table 1, arranged according to the sources of data, and convenient ways of pooling them. We can state directly that 16 of the 18 monohybrid ratios are entirely consistent with Mendelian theory, for the experimental deviation is relatively small in these 16 cases,—less than 1.9 times the probable error of the binomial distribution on the hypothesis of simple sampling. This means that we may expect a deviation as great or greater than the largest of the 16 about once in five times as a fluctuation of sampling. The exceptional cases are the ratio of agouti to non-agouti in depleted litters and possibly the ratio of dark-eyed to pink-eyed young from F_1 males. It is interesting to note in passing that the ratios show a deviation smaller than the probable error in nine cases, and larger in nine; and that the average of the sum of all eighteen quotients, $\frac{\text{deviation}}{\text{probable error}}$, is 1.25. We expect unity in the absence of any disturbances preventing the operation of pure chance. If we exclude the poorest ratio (with $\frac{\text{deviation}}{\text{error}} = 4.01$) and all totals because they are duplications, then the average of the 11 quotients for 11 distinct subgroups is 0.99.

LITTLE and PHILLIPS (1913) record a cross between a wild gray male and pink-eyed dilute brown females, in which a large F_2 (1180

TABLE I

Monohybrid ratios.

Allelomorphic pairs	Offspring obtained from	Frequencies		Calculated frequencies	Deviation	Probable error	Deviation / error
		Dominant	Recessive				
Agouti vs. non-agouti	F_1 ♀ ♀, (undepleted litters)	1118	1141	1129.5	11.5	16.03	0.72
	F_1 ♀ ♀, (depleted litters)	444	367	405.5	38.5	9.60	4.01
	F_1 ♀ ♀, total	1562	1508	1535	27.0	18.69	1.44
	F_1 ♂ ♂	214	200	207	7.0	6.86	1.02
	F_2 ♀ ♀	130	120	125	5.0	5.33	0.94
	Grand total	1906	1828	1867	39.0	20.61	1.89
Dark eye vs. pink eye	F_1 ♀ ♀, (undepleted litters)	1127	1132	1129.5	2.5	16.03	0.16
	F_1 ♀ ♀, (depleted litters)	412	399	405.5	6.5	9.60	0.68
	F_1 ♀ ♀, total	1539	1531	1535	4.0	18.69	0.21
	F_1 ♂ ♂	232	182	207	25.0	6.86	3.64
	F_2 ♀ ♀	127	123	125	2.0	5.33	0.38
	Grand total	1898	1836	1867	31.0	20.61	1.50
Black vs. brown	F_1 ♀ ♀, (undepleted litters)	1108	1151	1129.5	21.5	16.03	1.34
	F_1 ♀ ♀, (depleted litters)	402	409	405.5	3.5	9.60	0.36
	F_1 ♀ ♀, total	1510	1560	1535	25.0	18.69	1.34
	F_1 ♂ ♂	212	202	207	5.0	6.86	0.73
	F_2 ♀ ♀	120	130	125	5.0	5.33	0.94
	Grand total	1842	1892	1867	25.0	20.61	1.21
Total dominants vs. recessives		5646	5556	5601	45.0	35.69	1.26

individuals) was produced by mating the F_1 *inter se*. Their monohybrid ratios are as follows:

	Ratio	Deviation	Deviation / error
Agouti to non-agouti	894 : 286	9	0.90
Black to brown.....	923 : 257	38	3.79
Intense to dilute.....	894 : 286	9	0.90
Dark eye to pink-eye	915 : 265	30	2.99
Calculated 8.....	*885 : 295*		

Probable error = 10.033

They noted an excess of dominants in all of the four monohybrid ratios, although it may be added that the excess was quite surely not significant in three of them. Seven of the eighteen monohybrid ratios in our data show in contrast a slight but insignificant excess of recessives. There is evidently no necessary and fixed tendency to produce an excess of dominants such as LITTLE and PHILLIPS found. The ratios given for the sum of the depleted and undepleted litters or for the grand total in our data are comparable to the ratios given by LITTLE and PHILLIPS, for their data probably include both types of litters; but in two of these six ratios our data still show an excess of at least one recessive class, brown, whereas LITTLE and PHILLIPS record a striking and possibly significant deficiency of brown.

It is clear that a small but consistent excess in the dominant classes, such as LITTLE and PHILLIPS found, has a cumulative effect, so that in large numbers the excess of dominants, *irrespective of kind*, eventually becomes significant. Such is the case in the LITTLE and PHILLIPS data, for summing up the total of dominants and recessives, we find 3626 and 1094 respectively, which is a deviation of 86 from the mean value, 3540 : 1180. This deviation is 4.29 times the probable error, 20.07; hence, we must either regard the observed value as an infrequent fluctuation of chance (in fact too infrequent as it stands to be consistent with Mendelian theory) or we must admit selective elimination of recessives. The odds against a ratio as bad or worse are about 260 to 1. We find no such significant difference between total dominants and recessives in our data in table 1, for the ratio is 5646 : 5556, which gives a deviation of 45 from the calculated frequency, 5601. This deviation is only 1.26 times the probable error, 35.69; hence, we may conclude that the zygotes are formed in equal numbers and no marked general elimination of re-

cessives, irrespective of kind, has occurred between fertilization and classification, such as the data of LITTLE and PHILLIPS indicate. The only indication of any significant elimination of recessives occurs in the case of non-agouti in depleted litters and possibly in the case of pink-eyed offspring from F_1 males. The numbers in depleted litters are too small to affect the grand total. Nevertheless it would be unjustifiable to argue unreservedly from grand totals which obscure subgroups failing to agree with theory.

In order to compare the ratios of dominants to recessives derived from the various sources, and to study the effects of elimination, table I A has been constructed. Now if we take the totals from F_1 females

TABLE I A

The ratios of dominants to recessives, regardless of kind.

Offspring obtained from	Dominants	Recessives	Calculated	Deviation	Probable error	Deviation / error
F_1 ♀ ♀, undepleted litters (1)	3353	3424	3388.5	35.5	27.76	1.28
F_1 ♀ ♀, depleted litters (2)	1258	1175	1216.5	41.5	16.63	2.49
F_1 ♀ ♀, total (3)	4611	4599	4605	6.0	32.36	0.19
F_1 ♂ ♂ (4)	658	584	621	37.0	11.89	3.11
F_2 ♀ ♀ (5)	377	373	375	2.0	9.24	0.22
F_2 ♀ ♀ in tables 4 and 5 (6)	1162	1138	1150	12.0	16.17	0.74
Grand total	6808	6694	6751	57.0	39.19	1.45
Total of 2, 4, 5 and 6	3455	3270	3362.5	92.5	27.66	3.34

giving undepleted litters (no postnatal elimination) we obtain 3353 dominants to 3424 recessives, which is a deviation of 35.5 from the mean or calculated value 3388.5. There is, in fact, an excess of *recessives,* although not significant, for the probable error is 27.76. It is therefore clear that there is no selective prenatal elimination of recessives in the character pairs studied, that the zygotes are formed in accordance with theory, and that the very significant excess of dominants in the LITTLE and PHILLIPS data is probably due to postnatal selection. All of the ratios of table I A and the grand total of 6808 dominants to 6694 recessives are consistent with Mendelian theory, as is shown by the column, $\frac{\text{deviation}}{\text{error}}$. We may take the least favorable cases in which the most depletion has occurred by adding the results obtained in depleted litters from F_1 females to the ratios obtained in stock pens (ratios 2, 4, 5, and 6) in which a very large amount of elimination occurred. Even here

we have a ratio of 3455 dominants to 3270 recessives, and $\frac{\text{deviation}}{\text{error}} = \frac{92.5}{27.66} = 3.34$. The results are not a decisive test against a ratio of equality, although the ratio is not as good as might be desired to prove equality. With the exception of non-agouti, in depleted litters from F_1 females, a general elimination of recessives is not apparent in our experiments, and if it occurs at all, the selective value of a dominant factor is not marked. The excess of dominants in LITTLE and PHILLIPS's data may well have been due to elimination of recessives, for selection was undoubtedly more rigid than under our experimental conditions, where each pregnant F_1 female had a separate pen. A very large part of our total data came from such females as table 1 shows. The total from these F_1 females alone gives 4611 dominants to 4599 recessives,—remarkably close to the calculated value, 4605.

An examination of the results for each allelomorphic pair in table 1 shows the following:

Agouti vs. non-agouti

In all cases but one in our data, the ratios of agouti to its absence agree with the Mendelian expectation, and with the results shown in the experiments of LITTLE (1913) and LITTLE and PHILLIPS (1913). DURHAM (1911) obtained 76 agoutis to 37 blacks, and states: "The results should yield a ratio of 3:1; but my results are not in accordance with this ratio." However, the results are not far from the calculated 84.75:28.25, and may be regarded as a fluctuation due to sampling, when we compare the deviation, 8.75, with the error of a binomial distribution, 3.10.

We found 444 agouti to 367 non-agouti in our depleted litters, the calculated frequency being 405.5. This exceptional case stands out in contrast and suggests postnatal elimination of the non-agouti class. The actual deviation, 38.5, is over four times the probable error, 9.60; the odds against the occurrence of such, or greater deviations, being about 145 to 1. Since the undepleted litters show no excess of agouti (in fact they show an insignificant deficiency of agouti) the excess of agouti in depleted litters suggests selective postnatal elimination. This is also apparent if one examines the second column of table 3, bearing in mind that table 1 is derived entirely from table 3. Each agouti class is larger than the corresponding non-agouti class. Although the agouti factor in

conjunction with other factors gives the dull wild gray coat which is supposed to be of selective value as a protective color pattern, it is difficult to imagine how it can possibly be of any selective value under laboratory conditions unless it has other physiological effects or is closely linked with factors of selective value. However, the fact seems perfectly clear that litters depleted within the first two weeks of life show at that age an excess of agouti that is difficult to attribute to chance. The excess is not so large as to justify an unconditional conclusion, but deserves record. Further experiments will add data on this point. We may take for comparison the total results from all experiments in stock pens to see whether large numbers show agouti to be of selective value under the less favorable conditions in our experiments. There were 681 agouti to 698 non-agouti,—a result agreeing well enough with Mendelian expectation and yet showing an *excess of non-agouti.* Evidently the excess of agouti in depleted litters occurs only under the least favorable conditions where selection is rigid.

If we add to the total of table 1 the results from F_2 segregates bred back to pink-eyed brown males (recorded in tables 4 and 5), our grand total from all experiments shows a ratio of 2243 agouti to 2206 non-agouti, and a deviation of 18.5 from the calculated 2224.5. The deviation is actually less than the probable error, $22.49\left(\frac{\text{deviation}}{\text{error}} = 0.82\right)$ As far as we can tell, we are not including in this total any subgroups inconsistent with Mendelian theory except the case of depleted litters from F_1 females already noted.

Dark eye vs. pink eye

These two characters are no doubt alternative in inheritance, but all experimental data have not given results agreeing with a Mendelian hypothesis. The data of DURHAM (1911) and LITTLE and PHILLIPS (1913) agree closely with Mendelian theory. LITTLE (1913) published data showing a marked and significant deficiency of the pink-eyed form, both when the heterozygotes were mated *inter se* and when back-crossed to the recessive pink-eyed form. The results of the former cross were 1673 dark-eyed to 457 pink-eyed, which is a deviation of 75.50 from the mean value, 1597.5 : 532.5. The deviation is 5.60 times the probable error, 13.48. The odds against a ratio as bad or worse are 6200 to 1. LITTLE states that the results are more aberrant when the heterozygotes are mated back to the recessive form, but his data do not seem to justify

this conclusion. The results of this latter cross were 370 dark-eyed to 284 pink-eyed, a deviation of 43 from the mean value, 327. $\frac{\text{Deviation}}{\text{Probable error}} = 4.99$. The odds against a ratio as bad or worse are about 1300 to 1. LITTLE suggests some constitutional weakness in his strain of pink-eyed mice, either directly connected with the pink-eyed modification or closely associated with it, which tends to cause a higher mortality among the pink-eyed than among the dark-eyed young. However, selective elimination of the pink-eyed form is not indicated by our experiments, for the six ratios in table 1 give results agreeing closely with the calculated values. For example, in the 170 depleted litters from F_1 females with a total of 1120 young born, 811 lived to be classified, but the ratio of dark-eyed to pink-eyed shows a deviation from equality even less than the probable error. The widest deviation occurs in the young from F_1 males. In this case the ratio of 232 dark-eyed to 182 pink-eyed shows a deviation of 25. The $\frac{\text{deviation}}{\text{error}} = 3.64$. Although not a particularly good fit, it can hardly be said to be an impossible ratio. In view of the other ratios from stock pens showing elimination and the ratio from depleted litters, it is probably without special significance and does not indicate postnatal elimination of the pink-eyed form.

We may add to the total of table 1, the results found in tables 4 and 5 and obtain the grand total from all experiments. This gives a ratio of 2250 dark-eyed to 2211 pink-eyed and shows a deviation of 19.5 from the calculated 2230.5. The probable error is 22.52. The $\frac{\text{deviation}}{\text{error}} = 0.87$.

Black vs. brown

The factors for black and brown have been shown to be allelomorphic (LITTLE 1913, LITTLE and PHILLIPS 1913). Of the four monohybrid ratios given in the LITTLE and PHILLIPS data, the case of black and brown shows the greatest deviation from the calculated frequencies. The ratio of black to brown was 923 : 257, the calculated ratio being 885 : 295. The deviation, 38, is in this case 3.79 times the probable error, 10.03. The odds against the occurrence of such and larger deviations are about 92 to 1. While such a result is possible, it would in itself hardly demonstrate Mendelian inheritance in this particular cross as it stands.

All the ratios of black to brown in our subgroups and totals agree well with the calculated. There is no indication of elimination of brown; in fact, table 1 shows a slight excess. Adding to the total result of table 1 the ratios of black to brown in tables 4 and 5, we obtain 2315 : 2277, the calculated value being 2296. The deviation (19) divided by the probable error (22.85) is 0.83.

Summarizing, we may say that all monohybrid ratios for the three allelomorphic pairs studied are wholly consistent with Mendelian expectation, with the sole exception of agouti in depleted litters. With this exception noted, there is no suggestion in the subgroups or totals that postnatal or prenatal elimination vitiates the Mendelian ratios in individuals classified at the age of about two weeks. With respect to each allelomorphic pair, the gametes and zygotes are formed in equal numbers. There is no marked and necessary deficiency of recessives as a class, irrespective of kind, either in undepleted or depleted litters.

DIHYBRID RATIOS

The simplest ratio disclosing partial linkage is the dihybrid ratio; hence our data are arranged in the three possible dihybrid ratios in table 2. The fit of the observed ratios to the calculated is tested by PEARSON'S (1900) formula, $\chi^2 = \Sigma \frac{(o-c)^2}{c}$. The probable errors of χ^2 and P (PEARSON 1916) are not given, for we are not dealing with relative goodness of fit. In the original cross all the dominant characters were presented by one parent; and, if linkage exists, the parental combinations in the F_2 should be more frequent unless marked elimination occurs. Numerous dihybrid ratios have been recorded in mice and other mammals, but large numbers are usually obtained with difficulty.

We have rearranged LITTLE and PHILLIPS's data involving four allelomorphic pairs into the six possible dihybrid ratios in table 2 A. There are always some objections to pooling or subdividing data. In this study we are making the ratios tests against a Mendelian interpretation, and the data should bear dichotomous subdivision or the reverse process, provided no subgroups in the ultimate class frequencies show deviations so large as to exclude the same theory we are applying to the totals. This objection is seen, for example, in the excellent dihybrid ratio for agouti and intensity (table 2 A) taken from LITTLE and PHILLIPS's data, ($P = 0.818$). We are conscious of including in the 680 agouti intense, 436 wild grays (also agouti intense) which occur as one of the

four subgroups giving the total agouti intense. However this subgroup, wild gray, is so large that it is impossible to regard it as a wide chance deviation. In pooling their data to get the dihybrid ratio for agouti and intensity, we evidently are using wide negative and positive deviations which counterbalance each other. There is always danger of this sort in any Mendelian experiment. When we give totals we assume that, in the absence of more precise data, our subgroups have agreed tolerably well with theory. It is, of course, unjustifiable to manufacture a "good ratio" by the process of adding subgroups, some of which fail to agree with the same theory we apply to the total. We shall have to bear in mind that table 2 A has this objection, but it is interesting to note what we might have concluded had the cross been made for the sole purpose of studying any one of the groups of two allelomorphic pairs, regardless of the other characters involved. Mendelian crosses are often made in which many factors are unknown or disregarded,—our own data may very well be such a case.

There are no empirical probabilities in the cases of these dihybrid ratios, but *a priori* we can suppose each class should occur with equal frequency and we can test our result against this Mendelian hypothesis. The ratios studied are dealt with in the text in the same order as in table 2, as follows:

$$AD : Ad : aD : ad$$
$$AB : Ab : aB : ab$$
$$BD : Bd : bD : bd$$

Agouti and dark eye vs. non-agouti and pink eye

The cross, *AADD* × *aadd*, should, without linkage, give in the F_2 *AaDd, Aadd, aaDd* and *aadd* in equal numbers. The data from all sources in our experiments agree closely enough with such an expectation to suggest that the sets of deviations observed may well have arisen through sampling, if judged by the values of χ^2 and P. In no one of the 6 cases is the fit so poor as to preclude the hypothesis that the four kinds of gametes and zygotes are formed in equal numbers. There is, however, a peculiar relation in the arrangement of the class frequencies in the undepleted litters. It will be noted that the parental combinations (*AD* and *ad*) are nearly equal and yet smaller than the recombinations (*Ad* and *aD*) which are likewise nearly equal. The sort of maturation division which gives rise to *AD* is the same as that giving *ad;* i.e., these contrary class frequencies are complementary in a biological sense. *Ad*

Table 2

Dihybrid ratios.

Offspring obtained from	Class frequencies				Total	χ^2	P	Parental combinations	Recombinations	Calculated	Deviation	Probable error	Deviation / Probable error
	AD	*Ad*	*aD*	*ad*									
F_1 ♀ ♀, (undepleted litters)	530	588	597	544	2259	5.757	0.126	1074	1185	1129.5	55.5	16.03	3.46
F_1 ♀ ♀, (depleted litters)	225	219	187	180	811	7.520	0.058	405	406	405.5	0.5	9.60	0.05
F_1 ♀ ♀, total	755	807	784	724	3070	5.057	0.168	1479	1591	1535.0	56.0	18.69	3.00
F_1 ♂ ♂	116	98	116	84	414	6.986	0.072	200	214	207.0	7.0	6.86	1.02
F_2 ♀ ♀	66	64	61	59	250	0.464	0.908	125	125	125.0	0.0	5.33	0.00
Grand total	937	969	961	867	3734	6.911	0.075	1804	1930	1867.0	63.0	20.61	3.06
	AB	*Ab*	*aB*	*ab*									
F_1 ♀ ♀, (undepleted litters)	550	568	558	583	2259	1.074	0.784	1133	1126	1129.5	3.5	16.03	0.22
F_1 ♀ ♀, (depleted litters)	223	221	179	188	811	7.520	0.058	411	400	405.5	5.5	9.60	0.57
F_1 ♀ ♀, total	773	789	737	771	3070	1.870	0.602	1544	1526	1535.0	9.0	18.69	0.48
F_1 ♂ ♂	105	109	107	93	414	1.498	0.687	198	216	207.0	9.0	6.86	1.31
F_2 ♀ ♀	57	73	63	57	250	2.736	0.439	114	136	125.0	11.0	5.33	2.06
Grand total	935	971	907	921	3734	2.428	0.495	1856	1878	1867.0	11.0	20.61	0.53
	BD	*Bd*	*bD*	*bd*									
F_1 ♀ ♀, (undepleted litters)	539	569	588	563	2259	2.169	0.542	1102	1157	1129.5	27.5	16.03	1.72
F_1 ♀ ♀, (depleted litters)	215	187	197	212	811	2.549	0.473	427	384	405.5	21.5	9.60	2.24
F_1 ♀ ♀, total	754	756	785	775	3070	0.882	0.825	1529	1541	1535.0	6.0	18.69	0.32
F_1 ♂ ♂	122	90	110	92	414	6.754	0.082	214	200	207.0	7.0	6.86	1.02
F_2 ♀ ♀	66	54	61	69	250	2.064	0.561	135	115	125.0	10.0	5.33	1.88
Grand total	942	900	956	936	3734	1.829	0.612	1878	1856	1867.0	11.0	20.61	0.53

and *aD* bear the same relationship. The maturation divisions may be looked upon as follows:

A	a		A	a
D	d		d	D
530	544		588	597
Parental combinations	1074		1185	

In the derivation of χ^2 and P, we regard neither the signs nor the order in which frequencies occur; but in biological data the order may at least be suggestive as in this case where the same sort of maturation division gives rise to both members of a pair of complementary classes. We do not know of any precise method of measuring the significance of order in which frequencies or deviations occur. It may be worth while to point out that in using the formula $\chi^2 = \Sigma \frac{(o-c)^2}{c}$, we specify at the outset that a given system of calculated frequencies is to be set over against the observed set. Ordinarily the calculated frequencies are not permutable or only slightly so, but in a back cross of this kind, where theory calls for n equal classes, they are permutable in $n!$ ways. In other words, it is perfectly possible to obtain the same value of χ^2 and P with $4!$ ($= 24$) arrangements of these identical class frequencies. Had we obtained any one of these, we should have come to the same general conclusion in each case as to goodness of fit between theory and observation. Nevertheless, not all of these 24 arrangements prompt the same biological suggestion. The whole scheme happens to assume an order which lends itself to a special explanation in a biological sense, namely, maturation divisions of the various kinds are only approximately equal in this case. Out of the total 24 permutations, only 8 ($= 2^2 \cdot {}_2P_2$) would be as suggestive as the one we found.

There is another method of approach which points to the same conclusion. The total number of maturation divisions giving parental classes is 1074, and recombinations is 1185. . There is a deviation of 55.5 from the calculated 1129.5, which is 3.46 times the probable error, 16.03. The odds against such a deviation or wider ones are 51 : 1. We have always regarded these two allelomorphic pairs as perfectly independent, but when large numbers in undepleted litters are used, the results suggest that this is approximately true and only in a practical sense, but not absolutely. This is repulsion in one sense, and suggests slightly different frequencies in the various kinds of maturation

divisions. It may be added that the class frequencies show association = —0.10. The data suggest a gametic ratio of 1 : 1.1. CAROTHERS (1913) and WENRICH (1916) counted at random the cases where the accessory goes with the larger or smaller dyad of an unequal pair. Both investigators found 51.3 percent of the cases in which the larger went with the accessory and 48.7 percent with the smaller and the accessory together. The deviation from equality is not significant, but it is interesting to note that both investigators found a deviation in the same direction. If continued counts gave the same proportion, the deviation would eventually become significant. It is quite possible that Mendelian recombinations, and hence maturation divisions, do not differ from many other forms of common experience such as coin-tossing, dice-throwing, and the like, in which a slight bias is often found.

All the other subgroups fail to show the relation indicated by the undepleted litters, for elimination probably obscures the small amount of repulsion. If we add to the total data of table 2 the data from brown agouti females in table 4, we have a grand total for all experiments as follows:

AD	*Ad*	*aD*	*ad*	Total	χ^2	*P*
986	1018	1015	939	3958	4.068	0.255

The LITTLE and PHILLIPS data as they stand (table 2 A) agree with our totals in this case and are no decisive test against a Mendelian hypothesis. $\chi^2 = 5.615$, $P = 0.135$.

TABLE 2 A

The dihybrid ratios obtained by rearranging the ultimate class frequencies in LITTLE *and* PHILLIPS's *data.*

The pairs of dominant characters from male grandparent (*XY*)	Double dominant, *XY*	Single dominant, *Xy*	Single dominant, *xY*	Double recessive, *xy*	χ^2	*P*
Agouti and dark eye	700	194	215	71	5.615	0.135
Agouti and black	707	187	216	70	8.435	0.039
Black and dark eye	730	193	185	72	16.200	0.001
Agouti and intensity	680	214	214	72	0.915	0.818
Black and intensity	701	222	193	64	6.989	0.072
Intensity and dark eye	706	188	209	77	8.508	0.038
Calculated	*663.75*	*221.25*	*221.25*	*73.75*		

Agouti and black vs. non-agouti and brown

The cross *AABB* × *aabb* should, without linkage, give in the F_2 equal numbers of *AaBb*, *Aabb*, *aaBb* and *aabb*. The total data and all the

subgroups show, on the whole, a rather remarkably close fit to the calculated value, and therefore agree with Mendelian theory. There is no evidence of linkage, for the parental combinations and recombinations are very nearly equal. The depleted litters do not indicate selective elimination in our experiments.

If we add to the total data of table 2 the data from pink-eyed agouti females in table 4, we have a grand total for all of our experiments as follows:

AB	*Ab*	*aB*	*ab*	Total	χ^2	*P*
1600	1024	973	982	3979	1,527	0.681

LITTLE and PHILLIPS's data as they stand (table 2 A) show a set of deviations which do not exclude a Mendelian hypothesis, but are not a particularly good fit. $\chi^2 = 8.435$, $P = 0.039$.

Black and dark eye vs. brown and pink eye

The cross *BBDD* × *bbdd* should, without linkage, give in the F_2 equal numbers of *BbDd, Bbdd, bbDd* and *bbdd*. As in the preceding case, the total data and the subgroups show a close fit to the calculated values and are therefore consistent with the theory that these two pairs of independent Mendelian factors give four classes of gametes and zygotes in equal numbers. There is no indication of linkage in the biological sense for the parental combinations and recombinations are very nearly equal. The differences are very small when compared to the probable error and may well be chance fluctuations.

If we add to the total data of table 2, the data from black females in table 4, we obtain a grand total for all of our experiments as follows:

BD	*Bd*	*bD*	*bd*	Total	χ^2	*P*
1036	986	1027	1003	4052	1.534	0.679

The LITTLE and PHILLIPS data on this dihybrid cross do not agree with Mendelian hypothesis. The total set of deviations gives a value of $\chi^2 = 16.20$ and $P = 0.001$. There is an excess of the double dominant entirely too large to be attributed to chance. This excess is not due to the elimination of the double recessive, but apparently occurs at the expense of the recombination classes. The total number of double dominants, 730, shows a deviation of 66.25 from the calculated 663.75. The *a priori* probability for the double dominant is 9/16. Since the σ of a binomial distribution resulting from samples of 1180 individuals is 17.04 and the probable error is 11.49, the odds against deviations as wide or

wider than 66.25 are about 10,000 : 1. It is perfectly clear that if this experiment had been conducted to determine the dihybrid ratio for black and dark eye, we would no doubt have called this ratio a very poor fit. Black and dark eye show positive association (+0.19). This association is not due to gametic coupling as the data show, but rather to the survival of the black dark-eyed class and in particular the wild gray class which makes up a large part of this black, dark-eyed total. The wild grays are in great excess and other black dark-eyed classes are at least not markedly deficient in LITTLE and PHILLIPS's ultimate class frequencies.

LITTLE and PHILLIPS's data in table 2 A, as they stand, give the following results:

Agouti and intensity vs. non-agouti and dilution

The data show an excellent agreement with theory, for $\chi^2 = 0.915$ and $P = 0.818$.

Black and intensity vs. brown and dilution

The data show a set of deviations possible on the basis of a Mendelian hypothesis. $\chi^2 = 6.989$, $P = 0.072$.

Dark eye and intensity vs. pink eye and dilution

The data do not exclude Mendelian theory in this case, although the fit is not as good as could be desired. $\chi^2 = 8.508$, $P = 0.038$.

Summarizing, we may say that all three types of dihybrid ratios, regardless of the sources of our data, are consistent with Mendelian theory. The case of agouti and dark eye, however, strongly suggests that a slight repulsion exists between these two factors based upon different frequencies in the two types of maturation division involved. Prenatal elimination does not distort the ratios, but postnatal elimination may have a marked effect as is shown in some of the ratios obtained by rearranging LITTLE and PHILLIPS's data. The factors *B* and *D* (particularly in conjunction with *A* and *I* in the wild gray) are effective for survival in competition.

TRIHYBRID RATIOS

The ultimate class frequencies in our experiments are given in the trihybrid ratios in table 3, arranged according to the sources of data. These ratios describe the cross as a whole and were used to obtain tables 1, 1 A, and 2 by pooling class frequencies. The LITTLE and PHILLIPS data

have been rearranged in the four possible trihybrid ratios in table 3 A for comparison. As in the case of the simpler ratios, their experiment might have been made for the sole purpose of studying any one of these trihybrid ratios, and the conclusions would have varied according to the particular group of three characters chosen for study. We can state directly that the six ratios in our data are consistent with Mendelian expectation if judged entirely by the values of χ^2 and P. Two of the four trihybrid ratios in LITTLE and PHILLIPS's data are not consistent with a Mendelian expectation, for selective elimination has probably distorted the results. We again find that their ratios which group the black, dark-eyed classes together the most unsatisfactory.

TABLE 3

Trihybrid ratios

Color classes	Zygotic constitution	Offspring obtained from					Grand total
		F_1 ♀♀ (undepleted litters)	F_1 ♀♀ (depleted litters)	F_1 ♀♀ total	F_1 ♀♀	F_2 ♀♀	
Black agouti	*AaDdBb*	256	122	378	62	35	475
Brown agouti	*AaDdbb*	274	103	377	54	31	462
Black	*aaDdBb*	283	93	376	60	31	467
Brown	*aaDdbb*	314	94	408	56	30	494
Pink-eyed black agouti	*AaddBb*	294	101	395	43	22	460
Pink-eyed brown agouti	*Aaddbb*	294	118	412	55	42	509
Pink-eyed black	*aaddBb*	275	86	361	47	32	440
Pink-eyed brown	*aaddbb*	269	94	363	37	27	427
Total		2259	811	3070	414	250	3734
Calculated class frequencies		*282.375*	*101.375*	*383.75*	*51.75*	*31.25*	*466.75*
χ^2		8.039	11.047	6.774	10.116	7.536	10.626
P		0.330	0.137	0.454	0.183	0.377	0.157

There are some additional facts, however, which deserve detailed comment. As in the case of agouti and dark eye in the undepleted litters in table 2 the undepleted litters in our trihybrid ratio show a suggestive order or arrangement of frequencies. The contrary class frequencies which represent biologically complementary classes (e.g., *ADB* and *adb*) formed by the same sort of maturation division show some tendency to equality. It will be noted, for example, that black agouti (*ADB*) and pink-eyed brown (*adb*) are the two smallest classes. They are the result of the same sort of maturation division, for presumably every time an *ADB* egg is produced, an *adb* polar body is produced, and *vice versa*. The

brown agouti (*ADb*) and its complement, pink-eyed black, (*adB*) are the next largest classes and are nearly equal. The same general relationship exists between the remaining complementary classes. If we judge the whole system of deviations by χ^2 and P, we conclude that a fit as bad or worse is obtained about once in three times as a matter of random sampling. Since there is some suggestion of equality between complementary classes, one naturally wonders whether or not the various sorts of maturation divisions are only approximately equal, so that differences are not discovered except with very large numbers. We had noted, in the course of our experiment, as early as January, 1914, that the complementary classes, black agouti and pink-eyed brown, were the smallest and also equal. At that time 263 offspring in undepleted litters were classified, and the frequencies (given in the same order as in table 3) were as follows: 25, 33, 37, 33, 39, 28, 43, 25. We thought there was no special significance to these facts, although we were aware that our results differed from those of LITTLE and PHILLIPS, in whose data the black agouti class gave an excessively large *plus* deviation, whereas our black agouti class showed a *minus* deviation. Continuing the experiment, we had noted the same general tendency whenever a summary of results was made. Just as in the case of agouti and dark eye in the dihybrid ratios, we find that the order in which the frequencies occur is suggestive in a biological sense, although measuring the goodness of fit by χ^2 does not in itself reveal this fact. The case is made a little more striking if we use the class frequencies found shortly before the end of our experiment (January 1917). Given in the same order as in table 3, they were 256, 273, 283, 313, 294, 291, 275, 266. It is possible, of course, to obtain the same value of χ^2 and P with 8! (= 40320) permutations of these identical class frequencies. Had we obtained any one of these 40320 permutations, we should have come to exactly the same general conclusions in regard to the agreement between theory and observation. But it must be perfectly clear that not all of the orders would convey the same biological suggestion. In our particular case, the whole scheme happens to assume an order which is highly suggestive and lends itself to a special explanation. Now there are only 384 orders out of a possible 40320 ($2^n \cdot {}_nP_n$, where n = number of contrary pairs, 4 in this case) which would have been equally suggestive. It is what we might expect if the different types of maturation divisions were only approximately equal. We have always regarded these three allelomorphic pairs as absolutely independent, but when larger numbers than are usually available are used and the F_2 is obtained by back cross, the re-

sults suggest that this is only approximately true, but the assumption is probably sufficiently accurate for practical purposes. It may well be that the peculiar arrangement in our trihybrid ratio is due in a large measure to the peculiar relation between *A* and *D*, shown in the undepleted litters in table 2.

In addition to the trihybrid ratio from undepleted litters, the other five trihybrid ratios give sets of frequencies which on the whole agree fairly well with the calculated. These include the depleted litters, the young born in stock pens, and the totals. While these ratios do not preclude a Mendelian hypothesis, it is an open question whether we ought to test our ratios against Mendelian theory alone. We cannot be sure that an *a priori* probability with eight equal classes is a strictly correct assumption as our most reliable experimental data, the undepleted litters, have indicated. Let us replace the *a priori* probabilities of Mendelian theory by the empirical probabilities determined from actual experimental data, the undepleted litters. The numbers are certainly not as large as could be desired for such purposes, but are the best available. In this case the depleted litters now show a very poor fit, as might be expected, for $\chi^2 = 17.286$ and $P = 0.016$. The wild gray class is far too large, since it is the largest class in the depleted and the smallest in the undepleted litters against which we are now making our test.

We thought it worth while to pool our ratios taken from all pens showing elimination, all of which ratios agree well enough individually with Mendelian theory. Hence we added to the depleted litters the young from F_1 males and F_2 females, the latter two having their young in stock pens where considerable elimination occurs. This gives 1475 segregates born under conditions where stringent selection is operative. The distribution thus obtained is about as unsatisfactory from the point of view of Mendelian theory as are the results of LITTLE and PHILLIPS. Our data show $\chi^2 = 19.405$, and $P = 0.007$, while LITTLE and PHILLIPS's data show $\chi^2 = 19.906$, and $P = 0.006$. Now, if we make our distribution a test against the empirical probability based upon our undepleted litters, the fit is even worse; for $\chi^2 = 28.300$ and $P = 0.00016$. In all these cases the wild gray form is the most important contributory element to the poor fit.

It is clear that postnatal elimination distorts Mendelian polyhybrid ratios in mice, particularly ratios including the wild gray class in competition with other classes. The survival of the wild gray class in these experiments has some bearing on our views relative to protective coat color and natural selection. It is far in excess of expectation in pedi-

greed cultures in the laboratory, where coat color is not effective as a protective agent. The corollary is that the wild gray combination in mice (and other rodents?) would have persisted in competition in the wild state regardless of the protective dull gray coat color. We do not doubt that the wild gray coat is a protective color under some conditions, but the effect of the factorial combination, *ADIB*, is more far-reaching

TABLE 3 A

The trihybrid ratios obtained by rearranging the ultimate class frequencies in LITTLE *and* PHILLIPS'S *data.*

The three dominant characters from male grandparent (*XYZ*)	Mendelian classes								χ^2	*P*
	XYZ	*XyZ*	*XYz*	*xYZ*	*Xyz*	*xYz*	*xyZ*	*xyz*		
Agouti, black, and dark eye	566	134	141	164	53	52	51	19	19.906	0.006
Agouti, black, and intensity	539	141	168	162	46	54	52	18	9.082	0.247
Agouti, dark eye, and intensity	539	141	161	167	53	48	47	24	11.300	0.127
Black, dark eye, and intensity	563	138	167	143	55	42	50	22	20.822	0.004
Calculated	*497.81*	*165.94*	*165.94*	*165.94*	*55.31*	*55.31*	*55.31*	*18.44*		

than external appearances indicate. We have no reason to suspect that the combinations *ADB* or *ADIB* are more frequent gametic or zygotic combinations, as our undepleted litters show quite distinctly. In fact, the evidence points to the conclusion that the wild gray is one of the *least* frequent gametic recombinations. In the original LITTLE and PHILLIPS data 436 wild grays (*ADIB*) were observed, the calculated being 373.36. The deviation, 62.64, is 5.81 times the probable error, 10.78. The odds against deviations as wide or wider are about 12500 to 1. We wondered whether their data would show that the selective value of the wild gray class was apparently due to the effect of any combination of the four factors studied. If we consider as a class all individuals with any given two, three, or four factors, regardless of other differences, we find that the classes with *D* and *B* together are always far too large. Thus *DB*, *ADB*, *DIB*, and *ADIB* are all much larger than chance would possibly allow. The excess is due to the fact that any combination of factors with *D* and *B* in the ultimate class frequencies (*ADIB*, *aDIB*, *ADiB*, and *aDIB*) at least holds its own in competition, but the one class which shows the largest positive deviation is the wild gray, *ADIB*.

LITTLE and PHILLIPS classified their segregates at the age of about 4

weeks, while we classified ours at about two weeks, hence selection had been operative for a longer period in their experiments. Nevertheless our depleted litters show the same tendency as theirs. In our depleted litters the wild gray is the most frequent class, and shows the largest deviation from the calculated value. The deviation 20.625 is 3.25 times the probable error, 6.35. We cannot be sure that a test against Mendelian expectation is as justifiable as a test against the results of our undepleted litters. If thus tested the deviation is 31.09 which is 5.11 times the probable error, 6.09. If we add to the undepleted litters from F_1 females the results obtained in table 3 from F_1 males and F_2 females, which had young in stock pens where rigid selective conditions were present, we obtain 219 wild gray segregates out of a total of 1475. Tested against the Mendelian expectation, 184.375, we find a deviation of 34.625 which is 4.04 times the probable error, 8.57. Tested against our undepleted litters the deviation is 51.85, which is 6.31 times the probable error, 8.21. It is clear from the foregoing facts that the wild gray combination survives in competition even when the coat color and pelage are hardly developed; hence it is probable that it would have an advantage in natural selection regardless of protective coloration, although protective color would be an added advantage.

Summarizing the data on trihybrid ratios we may conclude that our ratios involving simultaneously agouti, black, and dark eye do not exclude a Mendelian hypothesis as they stand. There is, however, a peculiar order or arrangement in the undepleted litters which suggests strongly that complementary classes tend to be equal and that the four types of maturation division are only approximately equal. Grouping all experiments with depleted litters, we find a very poor agreement with the Mendelian expectation, and an extremely poor fit when tested against the empirical probabilities established by undepleted litters. Postnatal elimination distorts the ratios markedly, the wild gray class being the chief contributory element. The wild gray class survives in competition under laboratory conditions even at an early age and under conditions in which its protective coat color cannot function as such.

BREEDING TEST OF F_2 FEMALES

There were eight classes of F_2 segregates in our experiment. All classes were tested by breeding the females back to pink-eyed brown males, *aaddbb,* in stock pens. Of these eight classes, one is a trihybrid, three are dihybrids, three are monohybrids, and one is pure. The trihybrid, *AaDdBb,* has already been noted in the foregoing discussion and

TABLE 4

Breeding tests of F_2 dihybrid segregates from F_1 females in table 3, mated back to pink-eyed brown, aaddbb.

Class tested	Zygotic formula	F_3 classes expected				Total	χ^2	P	Parental combinations	Recombinations	Calculated	Deviation	Probable error	Deviation / error
Brown agouti	*AaDdbb*	*ADb*	*aDb*	*Adb*	*adb*									
		49	54	49	72	224	6.393	0.096	121	103	112	9.0	5.05	1.78
Black	*aaDdBb*	*aDB*	*aDb*	*adB*	*adb*									
		94	71	86	67	318	6.050	0.110	161	157	159	2.0	6.01	0.33
Pink-eyed agouti	*AaddBb*	*AdB*	*Adb*	*adB*	*adb*									
		65	53	66	61	245	1.710	0.639	126	119	122.5	3.5	5.28	.0.66

the results agreed qualitatively and quantitatively with Mendelian expectation.

The dihybrids (table 4) were brown agouti (*AaDdbb*), black (*aaDdBb*), and pink-eyed black agouti (*AaddBb*). Each dihybrid class gave 4 classes of F_3 segregates in expected proportions, for the results do not exclude a Mendelian interpretation, and do not suggest linkage.

The monohybrids (table 5) were brown (*aaDdbb*), pink-eyed brown agouti (*Aaddbb*), and pink-eyed black (*aaddBb*). Each monohybrid class gave two classes of F_3 segregates in the expected proportions.

TABLE 5

Breeding tests of F_2 monohybrid segregates from F_1 females in table 3, mated back to pink-eyed brown, aaddbb.

Class tested	Zygotic formula	F_3 classes observed		Calculated frequencies	Deviation	Probable error	Deviation / error
Brown	*aaDdbb*	*aDb* 84	*adb* 101	92.5	8.5	4.59	1.85
Pink-eyed brown agouti	*Aaddbb*	*Adb* 121	*adb* 125	123.	2.0	5.29	0.38
Pink-eyed black	*aaddBb*	*adB* 162	*adb* 133	147.5	14.5	5.79	2.50

The pure class, pink-eyed brown (*aaddbb*), bred true, giving 319 offspring.

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FLUCTUATIONS OF SAMPLING IN A MENDELIAN POPULATION[1]

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In experimental crosses with pedigreed cultures, the observed Mendelian ratios are supposed to be due to the chance combinations of 2^n kinds of gametes (n = number of allelomorphic pairs) occurring in equal numbers. The total or aggregate results may show larger or smaller deviations from the calculated ratios, but these deviations frequently lie within reasonable limits of error on the hypothesis of simple sampling. The aggregate results are obtained by adding subgroups—litters, families, pods, cobs, plants, or the like, in the experiments. The subgroups themselves may show extreme deviations. The theory of simple sampling which underlies strict Mendelism does not preclude such fluctuation in subgroups but calls for fluctuations of various sizes with *expected* frequencies. One could hardly postulate Mendelian inheritance with few or no deviations in subgroups or in totals. However, it is entirely conceivable that the total results in a Mendelian experiment may agree closely with the calculated ratio, and yet pure chance may not be operative, for extreme plus and minus deviations in the subgroups may counterbalance each other. YULE (1914) drew attention to such a case in analyzing LOCK's data on maize, for although the means agree very well with expection,

"the contrast between DR's selfed and the DR × RR crosses is curious. The former certainly do not suggest any significant fluctuation; the latter do on the whole suggest some source of disturbance or possibly error."

Mendel recognized wide fluctuations in the subgroups, and recorded in his original paper four plants which gave dominants to recessives in the ratios of 43 : 2, 32 : 1, 14 : 15, and 20 : 19. While such departures are not utterly impossible, their occurrence should be extremely infrequent. The odds against a ratio of 20:19, for example, are about

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1600 to 1. As one examines experiments recorded in the literature, one occasionally notes similar cases. HAYES and EAST (1915) record in table 1, a plant which gave 208 corneous to 142 floury kernels where 175 of each kind is the most probable expectation. The other 12 plants and the total in this table agree very well with theory, but the odds against this exceptionally wide deviation are about 2300:1. The fruit fly, Drosophila, used so commonly for demonstrating alternative inheritance, gives conspicuously poor ratios and wide deviations in sampling on a Mendelian hypothesis. In fact, it is almost impossible to speak of random sampling in this material. The numbers usually available in experiments with domestic animals are so small as to be misleading. One occasionally finds discrimination against bulls and stallions when a sire produces a large excess of one sex where the other is desired. In such cases the deviation from an approximate equality of both sexes is often very large but probably not impossible in relatively small samples, as fluctuations of sampling.

The object of this study has been to determine how closely deviations of various sizes in the subgroups, litters of mice, approach their expected frequencies in an actual breeding experiment, or whether such fluctuations have any significance other than chance. The results have proved serviceable as concrete illustrations of sampling in a Mendelian population.

The materials used in this study have been described in the preceding paper (DETLEFSEN and ROBERTS 1918). This paper deals only with the offspring of 90 trihybrid F_1 females (*AaDdBb*) bred back to ultimate recessive males (*aaddbb*). There were 561 F_2 litters, of which 361 were classified as undepleted and 170 as depleted. The undepleted are those in which the total number born was classified, but the depleted litters showed some elimination between birth and classification. I have made no attempt to subdivide the depleted litters according to the causes of elimination. Since there are three allelomorphic pairs involved in this cross we expect equal numbers of dominants and recessives with respect to any one pair. The results of the former study on this material agree with such an hypothesis. The chances should be even that any individual in the segregating generation will show agouti (*A*) or non-agouti (*a*); similarly black (*B*) or brown (*b*); and dark eye (*D*) or pink eye (*d*). Hence, we can study (1) the fluctuations of sampling in each one of these pairs separately, and (2) the fluctuations in dominants and recessives by disregarding the particular characters and classifying all characters simply as dominant or recessive.

If p is the probability that an event (such as the occurrence of a dominant) will happen in a single trial, and $q = 1 - p$ is the probability that it will fail to happen (when the allelomorphic recessive occurs), then ${}_nC_r\, p^r\, q^{n-r}$ is the probability that the dominant will happen exactly r times in n trials. The expression ${}_nC_r$ denotes, to be sure, the number of combinations of n things taken r at a time. For example, if we ask ourselves in a concrete case what is the probability of obtaining 4 blacks in a litter of 6, where the probability is ½ that any single individual will be black and ½ that it will be brown, then $p = q = ½$, $n = 6$, $r = 4$, and ${}_6C_4\ (½)^4\ (½)^2 = \frac{15}{64}$. That is, $\frac{15}{64}$ of our litters of 6 should show 4 blacks and 2 browns as the most probable expectation. The relative frequencies with which the dominant will happen r times in n trials, where $n =$ the size of litter and r has all values from 0 to n inclusive, are given by the expansion of $(q + p)^n$. Therefore the several probabilities that the individuals of a litter of any size, n, will show the dominant character 0, 1, 2, 3 ..... n times are $q^n + {}_nC_1 q^{n-1}\ p + {}_nC_2 q^{n-2}\ p^2$ ..... ${}_nC_{n-1}\ q\ p^{n-1} + {}_nC_n p^n = (q + p)^n = 1$. The calculated mean value of dominants for litters of any size, n, is np and the $\sigma = \sqrt{npq}$. In this paper $p = q = ½$ in all cases, for the F_1 was always mated back to the recessive type. Hence, the expected proportions of litters of 1 showing 0 and 1 black individual are ½ + ½; of litters of 2 showing 0, 1 and 2 blacks are ¼ + 2/4 + ¼; and so on. It is understood that we are making our results a test against a Mendelian or *a priori* probability, a course which is justified by the study of this same material in the previous paper. It would not be justified in monohybrid ratios in which the total results failed to agree with Mendelian expectation, as in the case of waltzing vs. normal gait in DARBISHIRE'S (1904) data. There are several ways in which the data may be treated, but a direct comparison of the experimental results with the calculated binomial distribution is the simplest and, it seems to me, most satisfactory. In all of the tables, the results are given in terms of the dominant character, but the results would be exactly the same if given in terms of recessives.

Table 1 records the frequencies with which the agouti, the dark-eyed, and the black-pigmented individuals occurred 0, 1, 2, ...n times in undepleted litters of various sizes. The litters ranged from 2 to 10 mice per litter. Italics indicate the calculated frequencies. The tendency for litters of all sizes to conform to expectation is clear in the case of each dominant character studied. It is equally true of their

recessive allelomorphs, since the observed distributions and calculated frequencies in terms of recessives are the same as those given for dominants, except that they stand in a reversed order. For example, in table 1, litters of 3, there are 1 + 5 + 3 + 2 litters with 0, 1, 2, and 3 dark-eyed, respectively. Stated in terms of the recessive pink-eyed, there are 2 + 3 + 5 + 1 litters with 0, 1, 2, and 3 pink-eyed, respectively, for manifestly if there are 5 litters of 3 with 1 dark-eyed in each, it follows that these 5 litters have 2 pink-eyed in each. The goodness of fit of the observed to the calculated frequencies is the same irrespective of the way in which the results are stated. In litters of 6, which are the most frequent, the fit is so close in all cases that we can conclude the deviations observed may well have arisen through sampling; for, using PEARSON's formula for goodness of fit, we find:

Agouti, $\chi^2 = 1.8069$ $P = 0.932$;
Dark eye, $\chi^2 = 5.3856$ $P = 0.507$;
Black, $\chi^2 = 5.3860$ $P = 0.507$.

HARRIS (1912) has shown the use and application of PEARSON's formula for goodness of fit in the case of Mendelian ratios.

It is not worth while to calculate the goodness of fit in the case of each litter size, because the total number of litters of the same size is too small in some cases. I have reserved this test to those cases in which the total litters of a given size are about 100 or more. However, we may add in the columns the frequencies of each attribute, and their calculated values, and thereby obtain the distribution of each type of dominant in the whole population. These totals represent the numbers of litters in which the dominants and recessives of the three kinds studied were equal, together with the total litters deviating from the theoretical mid-class value by +½, +1, +2 ..... +5, and by —½, —1, —2 ..... —5 dominants of each particular kind. It will be clear that in $(p+q)^n$ where n is odd, we have two mid-class values equally probable, but we cannot realize litters in such cases with an equal number of dominants and recessives; hence, I have kept these equally probable mid-class values separate where n is odd. The mid-class values in these cases really differ from a theoretical mid-class value or mean by —½ and +½. This in no way affects our totals. Applying PEARSON's test for goodness of fit of the totals observed to the totals calculated, we find that in undepleted litters of all sizes a fit as bad or worse is expected as a fluctuation of sampling 99 times in 100 for agouti, 66 times in 100 for dark eye, and 87 times in 100 for black. Hence we may conclude that our ex-

TABLE 1

Undepleted litters; fluctuations in number of agouti, of dark-eyed, and of black individuals in litters of various sizes.[2]

Size of litter	Classes	Number of agoutis, dark-eyed, and blacks in the litter													Total litters
		−5	−4	−3	−2	−1	Mid-class values		+1	+2	+3	+4	+5		
2	Agouti					1		4		2					7
	Dark eye					1		3		3					
	Black					4		3		0					
	Calculated					*1.75*		*3.5*		*1.75*					
3	Agouti					2	6		3	0					11
	Dark eye					1	5		3	2					
	Black					2	1		7	1					
	Calculated					*1.38*	*4.12*		*4.13*	*1.37*					
4	Agouti				0	9		8		5	1				23
	Dark eye				0	0		7		8	2				
	Black				2	5		9		7	0				
	Calculated				*1.44*	*5.75*		*8.63*		*5.75*	*1.44*				
5	Agouti				1	10	24		17	13	0				65
	Dark eye				1	11	24		16	8	5				
	Black				4	11	17		21	10	2				
	Calculated				*2.03*	*10.16*	*20.31*		*20.31*	*10.16*	*2.03*				
6	Agouti			1	8	19		28		25	11	1			93
	Dark eye			0	12	27		24		21	7	2			
	Black			0	5	22		28		25	10	3			
	Calculated			*1.45*	*8.72*	*21.80*		*29.06*		*21.80*	*8.72*	*1.45*			
7	Agouti			1	5	14	23		27	11	5	0			86
	Dark eye			0	3	21	24		23	11	4	0			
	Black			0	10	15	23		22	12	4	0			
	Calculated			*0.67*	*4.70*	*14.11*	*23.52*		*23.52*	*14.11*	*4.70*	*0.67*			
8	Agouti		0	1	7	10		18		11	5	1	0		53
	Dark eye		0	1	7	11		11		12	9	2	0		
	Black		0	2	5	13		18		7	6	2	0		
	Calculated		*0.21*	*1.66*	*5.80*	*11.59*		*14.49*		*11.59*	*5.80*	*1.66*	*0.21*		
9	Agouti		1	0	1	2	5		8	2	0	0	0		19
	Dark eye		0	2	0	2	4		7	2	1	1	0		
	Black		0	1	2	4	6		4	2	0	0	0		
	Calculated		*0.04*	*0.33*	*1.34*	*3.12*	*4.68*		*4.68*	*3.12*	*1.34*	*0.33*	*0.04*		
10	Agouti	0	0	0	2	1		0		0	0	1	0	0	4
	Dark eye	0	0	0	1	0		1		0	2	0	0	0	
	Black	0	0	0	0	1		2		0	0	1	0	0	
	Calculated	*0.00*	*0.04*	*0.18*	*0.47*	*0.82*		*0.98*		*0.82*	*0.47*	*0.18*	*0.04*	*0.00*	
Totals	Agouti	0	1	3	24	68	58	58	55	69	22	3	0	0	361
	Dark eye	0	0	3	24	80	57	46	49	67	30	5	0	0	
	Black	0	0	3	28	77	47	60	54	64	22	6	0	0	
	Calculated	*0.00*	*0.28*	*4.29*	*24.49*	*70.47*	*52.63*	*56.66*	*52.63*	*70.47*	*24.49*	*4.29*	*0.28*	*0.00*	

Totals { Agouti, $\chi^2 = 1.6836$, $P = 0.986$; Dark eye, $\chi^2 = 5.9115$, $P = 0.657$; Black, $\chi^2 = 3.7748$, $P = 0.875$

[2] In this and subsequent tables, the calculated frequencies were carried out three decimal places in obtaining the totals, and in calculating χ^2 and P.

TABLE 2

All litters, undepleted and depleted; fluctuations in number of agouti, of dark-eyed, and of black individuals in litters of various sizes.

Number classified in a litter	Classes	−5	−4	−3	−2	−1	Mid-class values			+1	+2	+3	+4	+5	Total litters
	Agouti						6		4						
	Dark eye						5		5						
1	Black						4		6						10
	Calculated						*5.00*		*5.00*						
	Agouti					2		9		6					
	Dark eye					2		9		6					
2	Black					7		5		5					17
	Calculated					*4.25*		*8.50*		*4.25*					
	Agouti					4	11		11	3					
	Dark eye					5	10		11	3					
3	Black					4	11		11	3					29
	Calculated					*3.63*	*10.87*		*10.88*	*3.62*					
	Agouti				1	17		17		14	2				
	Dark eye				1	10		22		14	4				
4	Black				4	12		25		8	2				51
	Calculated				*3.19*	*12.75*		*19.13*		*12.75*	*3.19*				
	Agouti				1	18	34		34	21	2				
	Dark eye				1	16	38		30	16	9				
5	Black				7	13	34		39	14	3				110
	Calculated				*3.44*	*17.19*	*34.37*		*34.38*	*17.19*	*3.44*				
	Agouti			1	9	30		33		38	14	2			
	Dark eye			0	16	30		32		27	10	3			
6	Black			2	7	30		36		33	16	3			127
	Calculated			*1.98*	*11.91*	*29.77*		*39.69*		*29.77*	*11.91*	*1.98*			
	Agouti			1	7	16	26		30	16	6	1			
	Dark eye			0	6	22	27		28	16	4	0			
7	Black			1	10	17	26		27	16	6	0			103
	Calculated			*0.80*	*5.63*	*16.90*	*28.16*		*28.16*	*16.90*	*5.63*	*0.80*			
	Agouti		0	1	7	10		21		12	6	2	0		
	Dark eye		1	1	7	12		14		13	9	2	0		
8	Black		0	2	6	15		19		9	6	2	0		59
	Calculated		*0.23*	*1.84*	*6.45*	*12.91*		*16.13*		*12.91*	*6.45*	*1.84*	*0.23*		
	Agouti		1	0	1	2	5		8	3	0	0	0		
	Dark eye		0	2	1	2	4		7	2	1	1	0		
9	Black		0	1	2	4	7		4	2	0	0	0		20
	Calculated		*0.04*	*0.35*	*1.41*	*3.28*	*4.92*		*4.92*	*3.28*	*1.41*	*0.35*	*0.04*		
	Agouti	0	0	0	2	1		0		0	1	1	0	0	
	Dark eye	0	0	0	1	0		1		1	2	0	0	0	
10	Black	0	0	0	0	2		2		0	0	1	0	0	5
	Calculated	*0.00*	*0.05*	*0.22*	*0.59*	*1.03*		*1.23*		*1.03*	*0.59*	*0.22*	*0.05*	*0.00*	
	Agouti	0	1	3	28	100	82	80	87	113	31	6	0	0	
	Dark eye	0	1	3	33	108	84	78	81	98	39	6	0	0	
Totals	Black	0	0	6	36	104	82	87	87	90	33	6	0	0	531
	Calculated	*0.00*	*0.32*	*5.20*	*32.61*	*101.69*	*83.34*	*84.68*	*83.34*	*101.69*	*32.61*	*5.20*	*0.32*	*0.00*	

Totals { Agouti, $\chi^2 = 2.9205$, $P = 0.938$; Dark eye, $\chi^2 = 2.8431$, $P = 0.942$; Black, $\chi^2 = 2.0802$, $P = 0.977$

perimental results are consistent with an hypothesis that litters are random samples of two kinds of zygotes of equal frequency in the case of each one of three allelomorphic pairs. Furthermore, since this is a back cross to the ultimate recessive, we may conclude that the gametes functioning at any ovulation are likewise random samples of two kinds in equal numbers, when we deal with a single allelomorphic pair.

YULE (1914) has studied the fluctuations of sampling in DARBISHIRE'S (1904) data on color and albinism in mice. There were 121 litters of various sizes in which $p = 3/4$ and 132 litters in which $p = 1/2$. Although the numbers in the few litters of each size are too small for an adequate test against (or close agreement with) the binomial distribution, the total populations agree very well in these two cases studied. YULE has so tabulated his data that litters of all sizes with the same number of albinos fall into columns and may be added. My data can be arranged in this form, if desired, directly from the tables presented. Nevertheless, there is an advantage in arranging the frequencies of litters of each individual size as in our tables so that the mid-class values, the deviations above, and the deviations below may be added separately. Even if there is only a slight tendency to intrafraternal correlation, good mean values or Mendelian ratios are obtained, but in the total population the ends of the frequency distribution are quite properly exaggerated. One can thus more easily recognize the cumulative effect of increased values above and below the mid-class value. I cannot imagine any force that would exaggerate the mid-class values themselves. In YULE'S tabulation the entire distributions of the smaller litters are included in the totals with the lower half of the larger litters. Furthermore, with YULE'S tabulation we obtain one set of values for χ^2 and P when the experimental results are given in terms of albinos, but a different set of values for χ^2 and P when the same experimental results are given in terms of colored individuals. For example, in YULE'S table A, $P = 0.94$, but given in terms of colored individuals, $P = 0.48$. That is, the total frequencies do not admit a reversed order. If we tabulate his table A as I have tabulated my results, $P = 0.77$, and this value is the same in terms of colored or albino individuals. The goodness-of-fit values in such a Mendelian population should be identical irrespective of whether we state our results in terms of the dominant or the recessive allelomorph.

Table 2 is the same as table 1, except that 170 depleted litters have been added to table 1, making a total of 531 litters which is a complete record of the entire experiment. The addition of depleted litters does

not modify our conclusions. The agreement in the totals between hypothesis and experiment is in fact remarkably close as is shown by the values of χ^2 and P for all three characters studied. The litters of 5, 6, and 7 occur over 100 times in each case, hence I have calculated χ^2 and P for each distribution separately (see table 2 A). On the whole, the values of P are as good as could be expected. The distribution of dark eye in litters of 5 shows the poorest agreement ($P = 0.038$), but does not show a very improbable set of deviations from the calculated, for such would occur about once in 25 times as a result of sampling. The small value of P is due in a large measure to 9 litters of 5 all of which showed dark eye, when only 3.44 were expected. Both the depleted and undepleted litters of 5 showed a slight excess in this case; but in view of the close agreement for dark eye in litters of 6 and 7, it is safe to regard this particular case as without significance other than a wide set of deviations due to sampling. The exceedingly close fit for agouti in litters of 7 is noteworthy ($P = 0.996$).

TABLE 2 A

The values of χ^2 and P in measuring the goodness of fit for litters of 5, 6, and 7 in table 2.

Number classified in a litter	Character	χ^2	P
5	Agouti	3.221$\frac{1}{7}$	0.667
	Dark eye	11.8361	0.038
	Black	5.9837	0.308
6	Agouti	4.9723	0.547
	Dark eye	4.9884	0.289
	Black	4.6457	0.591
7	Agouti	0.8315	0.996
	Dark eye	2.5903	0.761
	Black	3.5252	0.621

Each litter produced by an F_1 trihybrid female gave data on sampling with respect to three allelomorphic pairs. Each allelomorphic pair was treated separately in tables 1 and 2 in a test against calculated binomial distributions. By disregarding the particular characters and classifying a visible character as dominant or recessive, each litter becomes three separate samples of dominants and recessives. Treating the data in this way we obtain three times as many samples of an attribute in tables 3

TABLE 3

Undepleted litters; frequencies with which a dominant allelomorph occurred 0, 1, 2 - - - n times in the litters of various sizes. Calculated frequencies in italics.

Size of litter	Number of dominants in the litter												Total samples	Mean		Standard deviation	
	−5	−4	−3	−2	−1	Mid-class values		+1	+2	+3	+4	+5		Observed	Calculated	Observed	Calculated
2					6 *5.25*		10 *10.50*		5 *5.25*				21	0.95	1.00	0.72	0.71
3					5 *4.13*	12 *12.37*		13 *12.38*	3 *4.12*				33	1.42	1.50	0.85	0.87
4				2 *4.31*	20 *17.25*		24 *25.88*		20 *17.25*	3 *4.31*			69	2.03	2.00	0.93	1.00
5				6 *6.09*	32 *30.47*	65 *60.94*		54 *60.94*	31 *30.47*	7 *6.09*			195	2.48	2.50	1.14	1.12
6			1 *4.36*	25 *26.16*	68 *65.39*		80 *87.19*		71 *65.39*	28 *26.16*	6 *4.36*		279	3.09	3.00	1.22	1.23
7			1 *2.02*	18 *14.11*	50 *42.33*	70 *70.55*		72 *70.55*	34 *42.33*	13 *14.11*	0 *2.02*		258	3.35	3.50	1.28	1.32
8		0 *0.62*	4 *4.97*	19 *17.39*	34 *34.78*		47 *43.48*		30 *34.78*	20 *17.39*	5 *4.97*	0 *0.62*	159	4.00	4.00	1.38	1.41
9		1 *0.11*	3 *1.00*	3 *4.01*	8 *9.35*	15 *14.03*		19 *14.03*	6 *9.35*	1 *4.01*	1 *1.00*	0 *0.11*	57	4.19	4.50	1.50	1.50
10	0 *0.01*	0 *0.12*	0 *0.53*	3 *1.41*	2 *2.46*		3 *2.95*		0 *2.46*	2 *1.41*	2 *0.53*	0 *0.12*	0 *0.01*	12	5.17	5.00	1.82	1.58
Total Observed *Calculated*	0 *0.01*	1 *0.85*	9 *12.87*	76 *73.48*	225 *211.41*	162 *157.89*	164 *169.99*	158 *157.89*	200 *211.41*	74 *73.48*	14 *12.87*	0 *0.85*	0 *0.01*	1083	3.10 —	— *3.13*	1.24 —	— *1.25*

Total, $\chi^2 = 2.9187 \quad P = 0.938$

Table 4

All litters, undepleted and depleted; frequencies with which a dominant allelomorph occurred 0, 1, 2 - - - n times in the litters of various sizes. Calculated frequencies in italics.

Number classified in a litter	Number of dominants in the litter													Total samples	Mean		Standard deviation	
	−5	−4	−3	−2	−1	Mid-class values			+1	+2	+3	+4	+5		Observed	Calculated	Observed	Calculated
1						15 *15.00*		15 *15.00*						30	0.50	0.50	0.50	0.50
2					11 *12.75*		23 *25.50*		17 *12.75*					51	1.12	1.00	0.72	0.71
3					13 *10.87*	32 *32.63*		33 *32.62*	9 *10.88*					87	1.44	1.50	0.87	0.87
4				6 *9.56*	39 *38.25*		64 *57.37*		36 *38.25*	8 *9.56*				153	2.00	2.00	0.93	1.00
5				9 *10.31*	47 *51.56*	106 *103.13*		103 *103.12*	51 *51.56*	14 *10.31*				330	2.55	2.50	1.08	1.12
6			3 *5.95*	32 *35.72*	99 *89.30*		101 *119.06*		98 *89.30*	40 *35.72*	8 *5.95*			381	3.08	3.00	1.24	1.23
7			2 *2.41*	23 *10.90*	55 *50.70*	79 *84.49*		85 *84.49*	48 *50.70*	16 *16.90*	1 *2.41*			309	3.41	3.50	1.34	1.32
8		1 *0.69*	4 *5.53*	20 *19.36*	37 *38.72*		54 *48.40*		34 *38.72*	21 *19.36*	6 *5.53*	0 *0.69*		177	4.01	4.00	1.39	1.41
9		1 *0.12*	3 *1.05*	4 *4.22*	8 *9.84*	16 *14.77*		19 *14.77*	7 *9.84*	1 *4.22*	1 *1.05*	0 *0.12*		60	4.18	4.50	1.51	1.50
10	0 *0.01*	0 *0.15*	0 *0.66*	3 *1.76*	3 *3.08*		3 *3.69*		1 *3.08*	3 *1.76*	2 *0.66*	0 *0.15*	0 *0.01*	15	5.27	5.00	1.73	1.58
Total Observed *Calculated*	0 *0.01*	2 *0.95*	12 *15.61*	97 *97.83*	312 *305.07*	248 *250.01*	245 *245.03*	255 *250.01*	301 *305.07*	103 *97.83*	18 *15.61*	0 *0.95*	0 *0.01*	1503	2.89 —	— *2.89*	1.18 —	— *1.20*

Total, $\chi^2 = 1.4517 \quad P = 0.990$

and 4 as are found in tables 1 and 2 respectively, and we have what is tantamount to 1083 and 1593 litters in tables 3 and 4 respectively. The procedure is quite comparable to throwing simultaneously n coins of each denomination, a, b, and c, N times, in which we first measure the agreement between observed and calculated distributions for each denomination separately, and later pool the results as $3N$ samples of n coins regardless of denomination.

In table 3 are given the frequencies with which a dominant allelomorph occurred 0, 1, 2 .....n times in undepleted litters of various sizes. Table 4 is similar to table 3, except that both depleted and undepleted litters are included in table 4 and this table describes the entire experiment. In both of these tables it is clear that the experimental results agree closely with the calculated values, both in the case of the individual litter sizes and in the total populations, as is shown by the values of χ^2 and P.

The goodness of fit was calculated for the distributions of the more numerous litters of an individual size. The constants are recorded in table 5. The distribution of a dominant allelomorph in samples of 4,

TABLE 5

The values of χ^2 and P in measuring the goodness of fit for litters of 4, 5, 6, 7, and 8 in tables 3 and 4.

Number classified in each litter	Undepleted litters (table 3)		Undepleted and depleted litters (table 4)	
	χ^2	P	χ^2	P
4	2.6523	0.620	2.4944	0.648
5	1.2828	0.930	1.9753	0.852
6	4.5651	0.602	7.7116	0.262
7	6.7515	0.456	4.0193	0.777
8	1.5590	0.949	2.5600	0.955

5, 6, 7, and 8 agree as well with the calculated values as could be expected in a coin-tossing experiment or the like. Furthermore, the mean number of dominants in all samples of a given size (tables 3 and 4) shows close agreement with the calculated mean of the most probable distribution on the hypothesis of simple sampling. Although the probable errors of these means are not given, it is perfectly obvious that the difference between the observed and calculated means is small compared with the probable error of such a difference. Calculations were made in several cases and they agreed with this statement. The standard deviations of the distribution of litters of each individual size are likewise

given in tables 3 and 4. Like the means, the observed standard deviations agree closely with the calculated, and the difference between the observed and calculated values is exceedingly small compared with the probable error in every case. We may therefore conclude that fluctuations of a dominant zygote in litters of any size agree with our conception of sampling in a Mendelian population. It is also clear that the dominant and recessive gametes functioning at an ovulation are random samples of two kinds occurring in equal numbers. In Mendelian experiments we usually pool all samples and present observed ratios in totals, but if a more detailed analysis of our data is made, the subgroups agree with the theory of simple sampling of attributes which underlies strict Mendelism, for the fluctuations of various sizes approach closely their expected frequencies.

It has been shown (DETLEFSEN and ROBERTS 1918) that there is little or no evidence in our data, of selective elimination of recessives, particularly if our calculated values are based upon an *a priori* (Mendelian) probability. The fluctuations of sampling for dominants in *depleted* litters agree with this conclusion. I have given no table for this case, but one can be constructed readily from the data presented by subtracting table 3 from table 4, cell for cell. Such a table gives excellent agreement with the calculated values both in the individual litter distributions and in the total population. The goodness of fit for the distribution of dominants in depleted litters in the total population is shown by the value of $P = 0.850$.

It would be both interesting and valuable to examine the fluctuations of sampling in litters with respect to two or more allelomorphic pairs considered simultaneously, but sufficient data are not easily obtained; and, even if obtained, it is almost impossible to present them in satisfactory form. The number of possible types of litters is too large for tabulation. If, for example, we deal with two allelomorphic pairs, the relative frequencies with which segregating F_2 litters of any size, n, will show any one or any combination of the four zygotic types, *AB*, *Ab*, *aB* or *ab*, are given by $(p + q + r + s = 1)^n$ where p, q, r and s are the probabilities that *AB*, *Ab*, *aB* and *ab*, respectively, will happen in a single trial. The number of possible types of a given-sized litter is therefore ${}_{2a+n-1}C_n$, where $a =$ number of allelomorphic pairs, $2^a =$ number of zygotic classes, C stands for combinations of, and $n =$ size of litter. This is, simply stated, the number of combinations of 2^a kinds of different things taken n at a time, with repetition allowed. In this particular problem we require the number of terms in a complete homo-

geneous polynomial of the nth degree in 2^a variables. For example, in our experiment, litters of 6 were the most frequent, occurring 127 times. If we study the fluctuations of sampling for two allelomorphic pairs in such litters of 6, these litters may be distributed among 84 possible types, each type of litter being a different combination of the four zygotic classes. Not all types occur with equal probability, to be sure. With three allelomorphic pairs, these litters of 6 would show 1716 types. It will therefore be clear that a continuation of this study with two or more allelomorphic pairs is hardly possible with the numbers usually available.

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